# AUTOFLUORESCENCE SPECTROSCOPIC ANALYSIS OF SALIVARY METABOLITES IN PATIENTS WITH POTENTIALLY MALIGNANT DISORDERS, ORAL CANCER AND PATIENTS UNDER RADIOTHERAPY

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## CERTIFICATE BY THE GUIDE

This is to certify that **Dr. S.SENTHIL KUMAR**, Post graduate student (2014 – 2017 in the Department of Oral Medicine and Radiology **(Branch IX**), Tamil Nadu Government Dental College and Hospital, Chennai – 600003 has done this dissertation titled **"AUTOFLUORESCENCE SPECTROSCOPIC ANALYSIS OF SALIVARY METABOLITES IN PATIENTS WITH POTENTIALLY MALIGNANT DISORDERS, ORAL CANCER AND PATIENTS UNDER RADIOTHERAPY."** under my direct guidance and supervision for partial fulfillment of the M.D.S degree examination in April 2015 as per the regulations laid down by Tamil Nadu Dr.M.G.R. Medical University, Chennai -600032 for **M.D.S., Oral Medicine and Radiology (Branch – IX)** degree examination.

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## DECLARATION

| | |
|---|---|
| **TITLE OF DISSERTATION** | **"Autofluorescence spectroscopic analysis of salivary metabolites in patients with potentially malignant disorders, oral cancer and patients under radiotherapy"** |
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**CONTENTS**

**ABBREVATIONS:**

| | |
|---|---|
| OSCC | ORAL SQUAMOUS CELL CARCINOMA |
| OPMD | ORAL PREMALIGNANT DISORDERS |
| FAD | FALVIN ADENINE DINUCLEOTIDE |
| NADH | NICOTINAMIDE ADENINE DINUCLEOTIDE |
| PpIX | PROTOPHORPHYRIN IX |
| CPS | CYCLES PER SECOND |
| OSMF | ORAL SUBMUCOUS FIBROSIS |
| BQ | BETEL QUID |
| OL | ORAL LEUKOPLAKIA |
| OCs | ORAL CARCINOMA |
| LOX | LYSYL OXIDASE |
| TGF-β | TRANSFORMING GROWTH FACTOR |
| TIMPs | TISSUE INHIBITOR OF MATRIX METALLOPROTEINASES |
| COX | CYCLOOXYGENASE |
| IL | INTERLEUKINS |
| TNF-a | TUMOUR NECROSIS FACTOR |
| NO | NITRIC OXIDE |
| HNSCC | HEAD AND NECK SQUAMOUS CELL CARCINOMA |
| RNS | REACTIVE NITROSAMINE |
| ROS | REACTIVE OXYGEN SPECIES |

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## INTRODUCTION

Oral carcinoma (OC) account for more than 3% of all cancers diagnosed annually. Oral cancer (OC) is the commonest cancer in India, accounting for 50–70% of total cancer mortality[1]. OC is the sixth most common cancer worldwide. Two thirds of these patients have advanced stage disease at the time of initial diagnosis. Despite advances in treatment strategies, patients with late-stage cancers have a poor 5-year survival rate and a significant risk of treatment related morbidity[2]. Invasive squamous cell carcinoma (SCC) of the oral cavity is often preceded by various oral Potentially malignant disorders. The higher-grade OPMDs ( potentially malignat disorder with moderate dysplasia, severe dysplasia) have a greater likelihood of progression to invasive SCC. OPMDs detected in patients with a history of OC have an even greater risk of progression. Almost 4% of OC patients develop a second primary within 1 year of their initial treatment.The high risk group is older adult males who use tobacco and alcohol. The quality of life and survival of such high-risk patients is depends on detecting these OPMDs and OCs at an early-stage. Early diagnosis of OSCC can speed proceeding early intervention to treatment and can improve the prognosis[3].

Conventional oral examination (COE) is the standard method of revealing OPMDs and OSCC, confirming the clinical suspicion by biopsy. It is subject to interpretation of pathologists, and although it can detect cellular changes, it can only detect molecular changes if special techniques are employed. Currently available diagnostic technologies are histopathological examination, vital staining, biomarkers, DNA analysis, brush biopsy and optical techniques.

Early diagnosis is made possible with optical spectroscopy which will contain information about histological and biochemical characteristics[3] . The study is done to assess the

diagnostic utility of Laser induced Autofluorescence (LIF) spectroscopy by detecting metabolic and biochemical alterations in human saliva.

Interaction of light with tissues may highlight changes in tissue structure and metabolism. Optical spectroscopy systems to detect changes rely on the fact that the optical spectrum derived from a tissue will contain information about the histological and biochemical characteristics of that tissue.

The study was carried out using human saliva in differentiating the normal subjects from that of oral squamous cell carcinoma (OSCC), leukoplakia and Oral Submucous FIbrosis patients, (OPMDs) using the autofluorescence spectroscopy, altered spectral images due to the presence of endogenous porphyrins, NAD(P)H and FAD in the exfoliated cells from saliva proved in diagnosis of of both OPMDs and OSCCs. The elevated level of porphyrin in saliva of OSCC patients may be attributed to the disturbances in the amino acid degradation pathway and heme biosynthetic pathway, during the transformation of normal into dysplastic and malignant cells helped in diagnosis of the lesions[4]

**AIM:**

To evaluate the efficacy of Autofluorescence of saliva as an adjunctive diagnostic aid in premalignant and oral carcinoma patients and also to evaluate autofluorescence as a prognostic indicator in patients treated by radiotherapy.

**OBJECTIVES:**

1. To evaluate spectroscopic intensity of saliva in oral potentially malignant disorders.
2. To evaluate spectroscopic intensity of saliva in oral carcinoma patients
3. To evaluate spectroscopic intensity of saliva in control group and compare them with potentially malignant and oral carcinoma spectrum and to assess the difference in levels of fluorophores such as NADH, FAD, and PPIX levels as a tumour markers in saliva based on the spectrum obtained.
4. To evaluate spectroscopic intensity of saliva in patients under radiotherapy and compare with the intensity of oral carcinoma spectrum and thus evaluate the efficacy of autofluorescence as a prognositic indicator.

# REVIEW OF LITERATURE

## ORAL POTENTIALLY MALIGNANT DISORDERS:

The clinical concept of malignant transformation in oral mucosa has been proposed for more than 100 years. Sir James Paget first described malignant transformation of an oral lesion into tongue carcinoma in 1870. Schwimmer also reported the same finding in 1877[5]. The term "potentially malignant disorders" was defined by World Health Organization (WHO) oral cancer and precancer met in London in May 2005 the term 'potentially malignant disorders', as it conveys that not all lesions and conditions described under this term may transform to cancer, rather that there is a family of morphological alterations amongst which some may have an increased potential for malignant transformation. Potentially malignant disorders of the oral mucosa are also indicators of risk of likely future malignancies elsewhere in (clinically normal appearing) oral mucosa and not only site specific predictors as the risk of malignancy being present in a lesion or condition either during the time of initial diagnosis or at a future date[6]

## ORAL SUBMUCOUS FIBROSIS:

OSMF has been well established in Indian medical literature since the time of **Susrutha,** a renowned Indian physician, who described a condition resembling OSMF as *Vidari* under mouth and throat during the period of 2500-3000 BC. He noted pain on taking spicy food, depigmentation of oral mucosa and progressive narrowing of oral cavity[7].

**Schwartz (1952)**[8] for the first time reported a case of "Atrophia Idiopathica tropica mucosae oris" occurring in Indians in East Africa and described blanching and stiffness of the oral mucosa, difficulty in opening the mouth and inability to tolerate spicy food.

## EPIDEMIOLOGY:

**Pindborg JJ et al (1968)**[5] in their epidemiological survey concluded that there is an increased incidence of OSMF of about 0.18%-1.2% in urban population when compared with 0.04% - 0.4% in rural population.

**Ranganathan K et al (2004)**[9] conducted a study in Chennai, South India, reported a mean age of 32.4±10.4 years and median age of 29 years. The youngest and oldest ages of occurrence of OSMF in this study was 16 and 76 years in males and 24 and 57 years in females. Occurrence of the disease in individuals as young as 2, 3.5, 4, 11 and 12 years has also been reported.

## ETIOLOGY:

**Wahi PN et al (1966)**[10] in their study reported OSMF to be higher in patients with poor nutritional status. They found that the patients with OSMF showed a higher frequency of deficiency of vitamin A, B, C and multiple vitamin.

**Gupta PC et al (1966)**[11] stated that oral use of any tobacco product like gutkha contain arecanut and several other substances in powdered or granulated form which causes oral submucous fibrosis

**Van wyk CW et al (1988)**[12] have reported about irreversibility nature of the disease i.e., once OSMF induced by the habit of chewing betel nut, the reversal of the disease after cessation of the habit could not occur.

**Trivedy C et al (1999)**[13] in their study observed that copper is released from areca products during chewing and is deposited in oral tissues. They found that lysyloxidase activity is upregulated in OSMF patients.From these findings they hypothesized that cellular events lead to cross linking of collagen and elastin, making them less degradable. The upregulation of lysyloxidase in OSMF may be an important factor in the pathogenesis of this disorder.

**PRECANCEROUS POTENTIAL:**

**Paymaster JC (1956)**[14] was the first to mention submucous fibrosis as a premalignant condition. He reported transformation in 1/3rdof his patients with OSMF.

**Pindborg JJ (1972)**[15]gave the criteria for PML as

1. Higher prevalence of leukoplakia among OSMF patients.
2. Higher frequency of epithelial dysplasia.
3. Concurrent findings of OSMF in oral cancer patients.
4. Histopathological diagnosis of oral cancer without clinical suspicion among OSMF cases.
5. Higher rate of incidence of oral cancer among patients with OSMF

**Murti PR et al (1985)**[16] proposed that the malignant transformation rate has been reported to be 4.5% -7.6% over a 15 year period. Oral cancer developed 3-16 years after the diagnosis of submucous fibrosis. The average age at the time of malignant transformation was 64.6 years and age range was 48-81 years.

**PATHOGENESIS OF OSMF:**

Oral sub mucous fibrosis is a chronic disease and a well-recognized potentially malignant condition of the oral cavity characterized by inflammation and a progressive fibrosis of the lamina propira and deeper connective tissues[56]. Oral submucous fibrosis (OSMF) is also called as "diffuse oral submucous fibrosis', 'idiopathic scleroderma of mouth', 'idiopathic palatal fibrosis', sclerosing stomatitis', juxta-epithelial fibrosis',[57]

SIGNS AND SYMPTOMS ( figure 7)

• Progressive inability to open the mouth (trismus) due to oral fibrosis and scarring

• Oral pain and a burning sensation upon consumption of spicy foodstuffs

• Increased salivation

• Change of gustatory sensation

• Hearing loss due to stenosis of the eustachian tubes

• Dryness of the mouth

• Nasal tonality to the voice

• Dysphagia to solids (if the esophagus is involved)

• Impaired mouth movements (eg, eating, whistling,

blowing, sucking)

• Laboratory findings:

• Decreased hemoglobin levels

• Decreased iron levels

• Decreased protein levels

• Increased erythrocyte sedimentation rate

• Decreased vitamin B complex levels

**PATHOGOLGY OF OSMF**

Different hypotheses have been put forward so far in fully elucidating the pathogenisis of OSMF. The ***betel quid (BQ)*** chewing has been recognized as one of the important risk factors for OSMF as supported by the various experimental studies. The alkaloids and flavonoids from the BQ are absorbed and undergo metabolism which are the constant source of irritation to the oral mucosa during their contact. In addition, the fibres of areca nut also cause mechanical irritation to the oral mucosa which facilitates the diffusion of alkaloids and flavanoids into the subepithelial connective tissue, resulting in juxtaepithelial inflammatory cell infiltration[57].

Inflammation is characterized by the presence of activated T cells, macrophages and various chemical mediators. Persistent inflammation is crucial for the occurrence of tissue fibrosis. Thus, it can be considered that the induction of oral mucosal inflammation by BQ ingredients to be a critical event in the pathogenesis.

Growth factors like ***transforming growth factor- β (TGF-β)*** are synthesized at the sites of inflammation. At the molecular level, the collagen production and degradation are regulated by TGF-β and flavonoids present in areca nut[58].

**Collagen production pathway:**

The three main events which favours the collagen production are

1. Activation of procollagen genes
2. Elevation of procollagen proteinases levels
3. Upregulation of lysyl oxidase (LOX) activity

TGF-β activates the procollagen genes, resulting in the production of more pro-collagen. In OSMF, there is increased cross-linking of the collagen, resulting in increased insoluble form of collagen. The flavanoids also increase cross-linking in the collagen fibers.This is facilitated by increased activity and production of a key enzyme – LOX, which result in increased collagen production[59].

**Collagen degradation pathway:**

There are two main events regulated by TGF-β which decreases collagen degradation

1. Activation of tissue inhibitor of matrix metalloproteinases gene (TIMPs).
2. Activation of plasminogen activator inhibitor (PAI) gene.

TGF-β activates genes for TIMPs which inhibits the activated collagenase enzyme that is necessary for degradation of collagen. It also activates the gene for PAI, which is an inhibitor of plasminogen activator, results in absence of active collagenase. The flavanoids inhibit the collagenase activity. A reduction in the activity and levels of collagenase results in a decrease in collagen degradation[60].

***Lysyl oxidase (LOX)*** is a copper activated enzyme critical for collagen cross-linking and organization of extracellular matrix (ECM), which has been shown to be ten times more resistant to digestion by collagenase. A study was conducted to compare the LOX activity of fibroblasts derived from human normal mucosa and OSMF associated with betel nut

chewing. The study revealed that OSMF fibroblasts showed reasonably more lysyl oxidase activity than normal mucosa fibroblasts and this was statistically significant ($p<0.05$)[61].

***Copper*** also has been implicated in the pathogenesis of OSMF. Areca nut has been found to have a high copper content and play an important role in the pathogenesis of OSMF. The possible role of copper functioning as a mediator of fibrosis in OSMF has been proved by the finding that raised copper levels in oral biopsies from patients with OSMF[62].

OSMF is characterized by qualitative and quantitative alteration of collagen within the subepithelial layer of oral mucosa. The ***degradation of collagen by fibroblast phagocytosis*** is an important physiological remodeling of connective tissue. OSMF tissues exhibited 40% reduction of collagen phagocytic cells and a 48% decrease of fibronectin phagocytic cells as compared to normal fibroblasts. Normal fibroblast cultures incubated with areca nut alkaloids provided a dose-dependent reduction in the proportions of phagocytic cells. Thus inhibition of fibroblast phagocytosis by alkaloids provide a mechanism for the development of OSMF[63].

Further persistent tissue inflammation is thought to play a vital role on the occurrence of tissue fibrosis. The induction of oral mucosal inflammation by arecoline may be critical in the pathogenesis of OSMF. A study was conducted to compare role ***Cyclooxygenase (COX)-2*** expression in normal buccal mucosa and OSMF patients. The study found that COX-2 expression was significantly higher in OSMF specimens compared to normal buccal mucosa, however when the cells were treated with 80 μg/ml arecoline, COX-2 expression was upregulated in normal mucosa. These results concluded that the upregulation of COX-2 expression in human buccal mucosal fibroblasts could play a vital role in the pathogenesis of OSMF[64].

**Cytokines** play an important role in regulating proliferation, migration and matrix synthesis of fibroblast and it is the balance of these mediators which play a key role in regulating the initiation and progression of any fibrotic disease. TNF-α, IL-1, IL-6 and IL-8

have been implicated in the development of fibrosis. Conversely, IFN-γ is an antifibrotic cytokine and downregulation of which is seen in keloid and scleroderma patients[64].

A hypothesis that is commonly reported in OSMF patients is the ***epithelial alteration***. The epithelium is considered to be "atrophic" and therefore vulnerable to the effects of oral carcinogens. "Atrophy" is explained to be arise as a result of stromal changes, which include decrease in cellularity and vascularity with resultant tissue ischemia and undergoes progressive hyalinization[65].

Recent study with regard to vascularity in OSMF was conducted to assess the degree of expression of ***nitric oxide (NO)***, a net vasodilator, in OSMF. The study concluded that enhanced expression of inducible nitric oxide synthase (iNOS) noticed in OSMF mucosa. NO has diverse properties of angiogenesis, vascular dilatation and increased permeability of vessels. These properties are all contrary to the concept of tissue hypoxia in OSMF and therefore the proposed "ischemic atrophy" of the overlying epithelium. This augments the earlier contention of an alternative explanation for thinning of the epithelium often noticed in clinically advanced cases. The thinning may be attributed to the defective replenishment of the desquamated epithelial cell pool probably due to decreased proliferation of the adult stem cell. Based on this, hypoplasia being a more reasonable concept explaining epithelial "thinning" than that of "atrophy". The possible genotoxic and cytotoxic effects of NO on adult stem cells of epithelium and supporting stroma supports further impetus to this concept[67].

Regulation of ***transglutaminase-2 (TGM-2)*** by arecoline in oral fibroblasts have been found to play a major role in stabilizing the ECM proteins by cross-linking and making them highly resistant to protease degradation. [68]

Currently the pathogenesis of OSMF have focused on ***heme oxygenase-1 (HO-I)*** expression in fibrosis. HO-I, a microsomal enzyme, responsible for maintaining the cellular homeostasis. It plays an important protective role in the tissues due to reducing oxidative

injury and attenuating inflammatory response. HO-I is consistently and dramatically upregulated in a variety of fibrotic diseases, such as benign prostatic hyperplasia and cystic fibrosis of lung. OSMF demonstrated significantly higher HO-I mRNA expression than normal buccal mucosa on immnuhistochemistry. Arecoline was also found to elevate HO-I mRNA expression in a dose-dependent manner[69].

As OSMF produces changes localized to oral cavity, it has been put forth that ***saliva*** may have a role in the pathogenesis of OSMF. Saliva of OSMF patients have shown increased pH, increase in salivary amylase, increase in alkaline phosphatase and potassium, low level of calcium and normal level of salivary immunoglobulin. Formation of coagulum was observed in greater number of cases as the severity of the disease increased. It is thus postulated that the mechanical trauma due to chewing of betel nut, tobacco and chemical burns from slaked lime result in microhaemorrhage. The factor responsible for coagulum in saliva precipitates the increased laying down of fibroblast[70]. Increase in immunoglobulin levels is typically associated with three main chronic disease classes: those affecting the liver, collagen and chronic infections. The severity of OSMF was directly proportional to the estimated elevated levels of the major immunoglobulins IgG and IgA[70]. This raised levels of immunoglobulins being proteins can be detected by autofluorescence..

ORAL LEUKOPLAKIA:

DEFINITION:

Kramer (1978) had recognized the malignant potential of leukokeratosis and smokers patch and its relationship to pipe smoking[17]

Axéll (1996) states leukoplakia as a white patch measuring 5 mm or more which cannot be scrapped off and cannot be attributed to any other diagnostic disease[18]

First International Conference on oral leukoplakia. Malmo,Sweden defines as “A white patch or plaque that cannot be characterized clinically or pathologically as any other disease and is not associated with any physical or chemical causative agent except use of

tobacco[19]

The WHO (1997) described leukoplakia as "a predominantly white lesion of the oral mucosa that cannot be characterized as any other definable lesion[20].

In 2007 . *Warnakulasuriya et al* defines as "Leukoplakia should be used to recognize white plagues of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer" and stated that leukoplakia is a clinical term and the lesion has no specific histology. It may show atrophy or hyperplasia (acanthosis) and may or may not demonstrate epithelial dysplasia[21].

**Incidence and prevalence**

In the year 1992, Gupta *et al*. showed prevalence in various populations. In India, leukoplakia was found in 0.2% and 4.9% of the population present over 15 years of age[22]. Feller and Lemmer[23] estimated the prevalence of OL ranged from 0.5% to 3.46%, and also found the malignant transformation of OL from 0.7% to 2.9%.

Downer and Petti found an annual oral cancer incidence rate attributable to leukoplakia between 6.2 and 29.1 cases per 100,000 people[24]. Martorell-Calatayud *et al*. found the prevalence of OL ranges from 0.4% to 0.7% of the population[25]. Brouns *et al*. found the prevalence of OL is approximately 2% with an annual malignant transformation of approximately 1%.[26]

***Age and gender:***

Bánóczy (1977) found the incidence of malignant transformation as 5.8% in women and 2.1% in men, and the incidence was higher in patients with smokeless tobacco habit and alcohol, involved in the age-group of 51-60 years and sex distribution showed a male:female ratio of 3.2:1[27]

Espinoza *et al*. reported leukoplakia in all age groups with increased prevalence in the old population, ranging from 0.35% to 18.6%[28]

***Site specificity***

Axéll *et al*. (1996) studied site prevalence of OLs as- Buccal mucosa (76%), alveolar sulcus (19%), and tongue (5%)[28,29]. Schepman's et al (1998) showed vestibular mucosa as the most common site.[33] Brouns *et al* reviewed OL specified to eight sub-sites viz., Tongue, FOM, lower lip, hard palate, buccal mucosa, upper alveolus and gingiva, lower alveolus, and gingiva and finally in multiples sites[30].

**ETIOLOGY:**

Roed-Petersen *et al*. (1972) and Daftary *et al*. (1972) correlated the etiology of OL to *Candida* infection which was present in about 13.5% .The role of *Candida* in correlation with the clinical types and histological dysplasias was evaluated positively in the literature[32].

Bánóczy (1977)[33] observed statistically significant decrease in serum levels of Vitamin A, B12, C, beta carotene, and folic acid in patients with OL compared to controls. Schepman *et al*. found that smokers have 6 times higher risk of developing leukoplakia than non-smokers, despite lesions of non-smokers having a greater probability to evolve into cancer. Caldeira *et al*. (2011) found a high-risk factor of leukoplakia for malignant transformation is the infection with human papilloma viruses as the expression of oncogenic proteins such as human papillomavirus-16L1 can promote carcinogenesis[34]

Brzak *et al*. conducted study including 12,508 patients between 1998 and 2007 and found the highest frequency of leukoplakia in smokers.[34]

***Clinical types:***

Two main clinical types of leukoplakia are recognized, being homogeneous and non-homogeneous leukoplakia. The distinction of these is purely clinical, based on surface colour and morphological (thickness) characteristics, and do have some bearing on the outcome or prognosis. Homogeneous lesions are uniformly flat, thin and exhibit shallow cracks of the surface keratin. The risk of malignant transformation is relatively low. Nonhomogeneous lesions carry a much higher risk of malignant transformation

Non homogeneous varieties include: (figure 6)

• Speckled: mixed, white and red, but retaining predominantly white character.

• Nodular: small polypoid outgrowths, rounded red or white excrescences;

• verrucous: wrinkled or corrugated surface appearance.

Those with mixed white and red plaques should be recognized as having a higher risk status. These are to be denoted as "erythroleukoplakia"

proliferative verrucous leukoplakia (PVL) presents with multiple, simultaneous leukoplakias as the disease is visibly multifocal and frequently covers a wide area.

Warnakulasuriya et al gives "provisional diagnosis of leukoplakia is made when a predominantly white lesion at clinical examination cannot be clearly diagnosed as any other disease or disorder of the oral mucosa . A biopsy is mandatory. A definitive diagnosis is made when any aetiological cause other than tobacco⁄ areca nut use has been excluded and histopathology has not confirmed any other specific disorder"[21]

ORAL CARCINOMA:

Cancer is the second most common cause of death after heart diseases in developed coun-tries, and the third leading cause of mortality following heart and diarrheal diseases in developing countries.[35]

It is the 12th most common cancer in women and the 6th in men.[36] Almost 4–8.1% of females and 8–8.5% of males may develop oral cancer in their lives.[35] The habit of chewing betel nut leaves rolled with lime and tobacco, a mixture known as pan, results in prolonged contact of the carcinogen with the buccal mucosa, which is thought to be the principal cause of OC in India[28].

**Epidemiology :**

In 2012, the results of GLOBOCAN regarding oral cancer pointed out an incidence of 300,373 people worldwide, with an estimated rate of 5.5 in men and 2.5 in women each 100,000 people.

The estimated prevalence was 702,373 during the last 5 years, with a rate of 1.8 in men and 1.2 in women each 100,000 people. On the other hand, the mortality for malignant neoplasia of the oral cavity was 145,326 with an estimated rate of 2.7 in men and 1.2 in women[37]. In the oral cavity, the most frequent group of tumors are of epithelial origin, specially the squamous cell carcinoma (SCC), which are observed in 9 out of 10 neoplasias. The prevalence of SCC is about 95% of all types of oral cancer and during the past decade its incidence increased by 50%.[37]

**Etiology and Major risk Factors**

Numerous risk factors or possible causative agents for OC have been described. Chemical factors like tobacco and alcohol, biological factors like human papilloma virus (HPV), syphilis, oro-dental factors, dietary deficiencies, chronic candidiasis and viruses have been shown to be significantly associated with OC[38].

**Chemical Factors**

***Tobacco***

There are ample evidences suggesting that tobacco in various forms, including smoking, chewing and in betel quid etc., have carcinogenic impact in oral cavity. The commonest form of tobacco use is smoking. The various forms in which tobacco is used as smoke are- cigarettes, cigars, pipe and bidi etc. Hookah or chillum (a clay pipe used to keep the burning tobacco)[38]

***Alcohol***

Numerous studies have suggested alcohol to be a major risk factor for OC. There is a certain degree of controversy whether alcohol alone may have carcinogenic impact. This is due to simultaneous tobacco and alcohol intake of study subjects in various epidemiological studies. Studies have shown that individuals consuming more than 170 g of whisky daily have ten times higher risk of OC than the light drinkers . Alcohol may have additive effect and it has been suggested that it facilitates the entry of carcinogens into the exposed cells,

altering the metabolism of oral mucosal cells . However, the current evidences do not suggest that pure ethanol alone is carcinogen for the development of OC.[39]

***Viruses***

Role of oncogenic viruses in human cancer is an emerging area of research. HPV has been identified in approximately 23.5%.HPV-16 has been demonstrated in 90–95% of all HPV positive HNSCC cases, followed by HPV-18, HPV-31, and HPV-33. The prognostic significance of HPV in pre-cancerous oral lesion is not clear. Kassim et al also reported oncogenic relationship between HSV-1 and oral squamous cell carcinoma (OSCC)[40,41]

***Syphilis:***

The data on causal association between syphilis and OC is weak. There are reports of 19 and 6% serological positivity for syphilis among tongue cancer patients[40,41]

***Candida:***

Candida has been suggested to play a role in initiation of OC Clinical studies have reported that nodular leukoplakia infected with Candida has a tendency for higher rate of dysplasia and malignant transformation[40,43]

***Dental Hygiene and Related Factors:***

There is inverse association between oral hygiene and incidence of OC. Poor oral hygiene and prolonged irritation from sharp teeth have been viewed for their possible role in the development of OC. Poor oral hygiene and dental sepsis is thought to promote carcinogenic action of tobacco[44]

***Nutritional Factors***

Dietary deficiencies are also suggested to play a role in the development of OC. Some workers have reported lower risk of OC with higher intake of fruits and vegetables[45]

**ORAL CARCINOGENESIS:**

Oral carcinogenesis like any other cancer is a progressive disease and normal epithelium passes through stages starting from dysplasia to finally transforming into invasive

phenotypes. Use of genetic and proteomic approach in recent years have revealed the molecular pathological picture of OC[71].

***Genetic Susceptibility***

It is now established that up to 10% of all cancers have a strong hereditary component. Role of genetic component in the development of OC is being suggested by several studies showing familial clustering. Glutathione S-transferase M1 (GSTM1) null genotype appears to be the most consistent polymorphic susceptibility marker for head and neck cancer including OC. Meta-analyses by Tripathy and Roy showed that the GSTM1 null genotype conferred a 20–50% significantly increased HNSCC risk. The variant val allele of the CYP1A1 (Cytochrome P450, family 1, member A1) polymorphism is another fairly consistent susceptibility marker with a 35% increased risk in a meta- analysis of 12 studies. Brennan et al.ALDH2 (Aldehyde dehydrogenase 2) genes were associated with HNSCC[28,72].

***Proto-oncogenes, Oncogenes and Genetic Alterations***:

Genetic alterations define molecular basis of carcinogenesis which includes point mutations, amplifications, rearrangements, and deletions.

Epidermal growth factor receptor (EGFR), K-ras, c-myc, int-2, Parathyroid adenomatosis 1 (PRAD-1) and B-cell lymphoma (bcl) like oncogenes have been implicated in OC development [24]. Transforming growth factor-alpha (TGF-a) is known to promote neovascularization and mitogenesis. Loss of chromosome 17p is also frequent in most human cancer including OC. It is seen in approximately 60% of invasive lesions. Although p53 inactivation correlates closely with loss of 17p in invasive lesions, p53 mutations are quite rare in early lesions that contain 17p loss. Loss of chromosome arm 10 and 13q are also noted in primary tumors[28,73]

***Genomic Instability:***

Genomic instability such as loss of hetrozygosity (LOH) and microsatellite instability (MSI) are frequently observed in cancer. Chromosome 9p21 containing p16 tumor suppressor

gene is frequently lost in HNSCC and oral preneoplastic lesions. Chromosome 3p14 contains the tumor suppressor gene fragile histidine triad (FHIT) as well as a common fragile site, FRA3B which is also found to be frequently deleted in early tumorigenesis[74] and its deletion is associated with exposure to cigarette smoke. Loss of function of the tumour suppressor p53 can result in uncontrolled cell division and progressive genomic instability[75].

Nunes et al performed a microsatellite analysis of cells sampled from the oral cavity of oral and oro-pharyngeal cancer patients and observed LOH in 84% of samples. Spafford et al. identified genomic alterations in all of the malignant lesions of the oral cavity[28].

***Epigenetic Alterations :***

The major epigenetic modification in tumours is methylation. Changes in the methylation patterns can play an important role in tumorigenesis which leads to loss of genetic expression and important for the multiple indispensable genetic events during carcinogenesis. Malignant progression takes place because these alterations can inactivate DNA repairing genes.

Methylation patterns of p16, methylguanine-DNA methyltransferase (MGMT) and Death-associated protein kinase (DAP-K) genes in smears of patients suffering from head and neck cancer showed abnormal hypermethylation patterns by a methylation specific polymerase chain reaction (PCR)[76].

***Molecular Progression Model***

Califano et al. tested ten most common allelic events in a large number of primary pre-invasive lesions and invasive HNSCC to develop a molecular progression model. It involves inactivation of many putative suppressor gene loci. Chromosomes 9p and 3p appear to be lost early, closely followed by loss of 17p. Mutations in p53 gene are seen in the progression of pre-invasive to invasive lesions[77].

***Molecular Epidemiology***

The pattern of specific gene mutation in OC patient may give a clue to the aetiology of that particular tumor. Brennan et al analyzed the pattern of p53 mutation in HNSCC. They found that the incidence of p53 mutation was much higher in patients who were exposed to both tobacco and alcohol versus non-users. It has been suggested that alcohol appears to augment the effect of smoking due to an increase in the absorbance of carcinogens contained within the cigarette smoke.

## AUTOFLUORESCENCE IN DIAGNOSIS

Early detection of mucosal lesions can be enhanced by the use of a dilute acetic acid rinse and observation under a chemi- luminescent light (ViziLite). Epstein JB et al showed in his study were 78 of 100 patients who presented for dental screening and were examined by COE (under incandescent light) before and after a 1-minute rinse with 1% acetic acid, and then once again using the ViziLite, had clinically diagnosable benign lesions (*e.g.* linea alba, leukoedema) and 29 clinically undiagnosable lesions initially, but after the acetic acid rinse, six additional diagnosable lesions (linea alba) and three undiagnosable lesions were found. Study[46] Rosin et al showed that ViziLite revealed occasional lesions not seen under incandescent light[47].

Jayachandran et al(2009) showed that tissue autofluorescence and diffuse reflectance spectroscopic analysis of premalignant and malignant lesion was a Fast and noninvasive, diagnostic techniques tapping the potential to link the biochemical and morphologic properties of tissues for accurate diagnosis[48].

Jayachandran et al(2009) studied autofluorescence intensity of pre and post treated patients with oral submucous fibrosis and reported variations in the intensity of fluorescence at 385nm and 440nm intensity region corresponding to NADH. The average fluorescence spectrum of the post treated OSMF mucosa had a lesser intensity around 385 nm and a higher

intensity around 440 nm than that of the pre-treated OSMF mucosa, thereby mimicking the normal oral mucosa.[80]

Jayachandran et al (2016) studied Raman spectroscopy of saliva, urine and serum( a noninvasive inelastic light scattering technique in which the wavelength of the incident laser light shifts depending on the vibrations of the molecules). The specific biochemical, structural and conformational changes occurring in tissues is reflected by their Raman spectra well before the clinical manifestations start thus aiding in diagnosis and speedy treatment planning[49].

The application of lasers emanated from photodynamic therapy, a technique for cancer treatment has further expanded its ability to induce Autofluorescence had taken a further step in detection of malignant lesions[50]. In the late 1970s, it was discovered that autofluorescence (also called natural or endogenous fluorescence),which had until then been regarded only as a disturbing background signal in exogenous fluorescence detection, could be used for cancer detection as well[51]

Wang cy et al showed Light-induced fluorescence spectroscopy can distinguish between benign (normal and hyperkeratosis) and dysplasia with a sensitivity of 92% and a specificity of 95%[52]. Zhang et al Laser-induced fluorescence spectroscopy used to examine OSCC in the hamster buccal pouch model shows increased fluorescence in malignant areas[53]

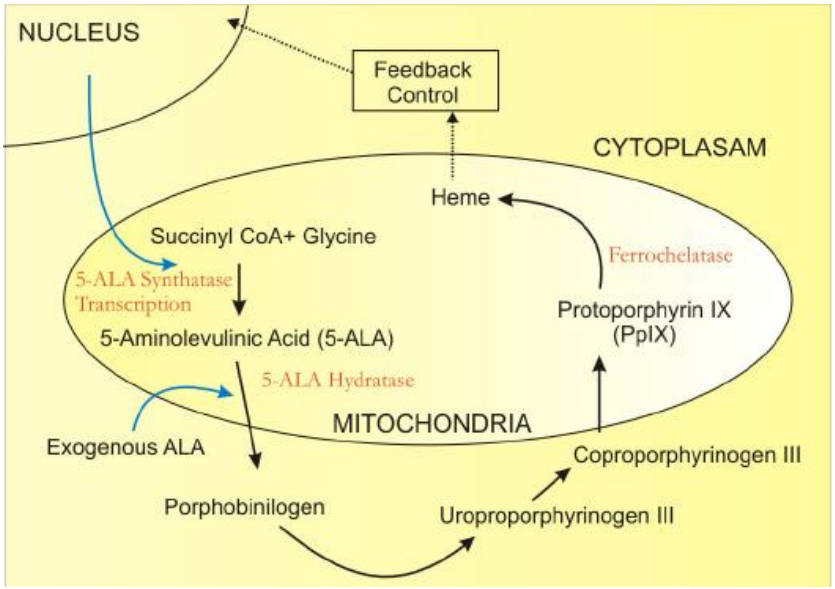
Zheng and Leuing et al studied in Another light based study by Fluorescence photography detected OSCC with a sensitivity of 91% and specificity of 85%. The relatively low sensitivity and specificity of auto-fluorescence can be markedly improved by adding an exogenous chemical such as aminolevulinic acid (ALA)[54,55]

Recently, diagnosis based on saliva, picked up much importance in the screening of various diseases as its collection was very simple and easy, when compared to the collection of blood and also, any physiological and pathological changes in the body will be reflected in

saliva. In oral carcinoma and PMDs, there were considerable changes in the DNA, oxidative protein in saliva, due to reactive nitrosamine (RNS) like nitric oxides (NO), nitrites (NO2) and nitrates (NO3) and reactive oxygen species (ROS) such as hydrogen peroxide (H2O2), oxygen($O_2^*$) and hydroxyl ($OH^*$) free radicals[4] .

Systemic alterations in metabolic and pathological conditions bring about changes in the distribution and the photochemical characteristics of key amino acids, proteins, enzymes and endogenous porphyrin etc. Protoporphyrin (PpIX) is an endogenous porphyrin that is associated with tissue alterations and malignancy. This porphyrin gets selectively accumalted in abnormal tissue due to a break in the heam cycle. Therefore, the relatively narrow porphyrin fluorescence peaks from PpIX could be informative about the condition of the mucosa under investigation. The elevated level of porphyrin in tissues due to altered heme biosynthetic pathway and/or amino acid degradative pathway results in excess amount of porphyrin on tissues may create oxidative stress, and may be released out dysplastic and malignant cells through pinocytosis.(figure 11) Hence, biofluids of oral cancer patients may have elevated level of Porphyrin. These alterations can be detected by the spectrometer and the spectral changes can be utilised to acertain the diagnosis[81].

FIGURE 11



## PRINCIPLE OF AUTOFLUORESCENCE

Photoluminescence is the physical process of light emission from any substance that has not been heated and takes place from the electronically excited states. Depending upon the nature of the excited state, luminescence can be strictly divided into two types, fluorescence and phosphorescence. Fluorescence is a rapid and spin-allowed emission of light from singlet excited states, while in phosphorescence emission of light comes due to spin-forbidden transitions from triplet excited states to the ground state**.**

Autofluorescence is produced by fluorophores that naturally occur in living cells after excitation with a suitable wavelength. The fluorophores are located in the tissue matrix or in cells (e.g. collagen, elastin, keratin and NADH, Phorphyrins (PPIX).(table 1) The changes in the concentrations and microenvironments of fluorophores alter tissue fluorescence to a sufficient extent to detect metabolic and pathological changes related to precancerous and cancerous growth. The presence of disease changes the concentration of the fluorophores .

The physiopathological concept that explains tissue autofluorescence response is based on changes in the cell structure such as hyperkeratosis, hyperchromatin, increase in pleomorphism, variations in nucleus size and even cell volume and metabolism concentration of flavin adenine (FAD) and nicotinamide adenine dinucleotide(NAD) in epithelial and in subepithelial stroma. In particular, these changes in the epithelium and in the stroma, may alter the distribution of the fluorophores in the tissues and consequently the way by which the fluorescence is emitted after been stimulated by the visible light[82]

Hemoglobin strongly absorbs the autofluorescent light produced by collagen and elastin. More specifically, the increased presence of submucous blood associated with oral cancer and its angiogenesis process may enhance the absorption of collagen and elastin produced by the autofluorescent light, this is why the area may appear darker during the examination[83]

***Autofluorescence Due To Fluorescent Coenzymes:***

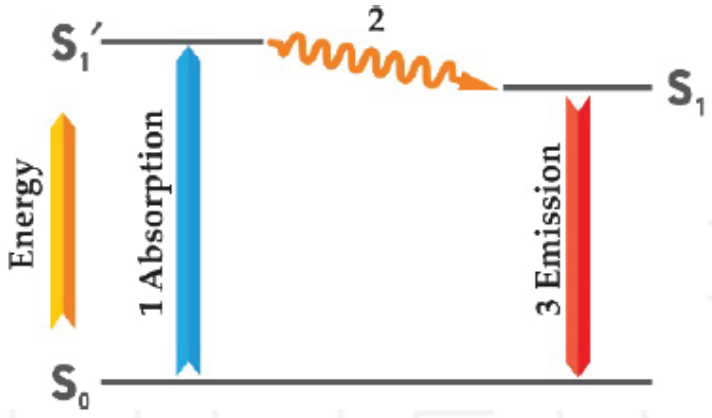
The coenzymes NADH and NADP(H), located mainly in the mitochondria of cells, act as hydrogen transferring molecules in the respiratory chain. The oxidized forms NAD and NADP fluorescent about 445nm. However, the fluorescence yield of the oxidized form is 1000-foldlessthanthereducedforms (for excitation >300nm).Therefore, in practice, UVA excited *invivo* fluorescence can be attributed to NADH/ NADPH.

The fluorescent coenzymes act as sensitive bioindicators of metabolic functions such as the degradation of glucose or respiration. In particular, NADH may serve as an indicator of the intracellular oxygen concentration (table 1) shows various fluorophores with their excitation and emission maxima (.B.Chance *et al.*(1962)*Science*137,499-508).

**Principle of fluorescence**:

Fluorescence is typically the emission of light from aromatic molecular depending on excited states. Fluorescence is the luminescence in the molecular which absorb a photon to a higher energy state (the excited state). This state triggers the emission of another photon with a longer wavelength. The electron in excited orbital is paired to the second electron in the ground state orbital. When returning to the ground state it is spin allowed and occurring rapidly by emission of a photon .(figure 2)

**FIGURE 11**



A fluorophore will not fluoresce in its stable configuration (ground state or a relatively low-energy state). Since the fluorophore is unstable at high-energy configurations, it will reduce to the lowest energy excited state by undergoing several non-radiative deactivation processes (semi-stable) then decays back to the ground state and release or emitted the excess energy as light. The lifetime of fluorescent states is approximately 10−5 to 10−10 s. The fluorophore can absorb light energy again and repeat the entire process. The energy of the fluorescence photon is lower than the energy of the absorbed excitation photon generally due to the radiative loss in the excited state. Thus, the wavelength of the fluorescence is longer than that of the excitation. The magnitude of the Stokes shift is determined by the chemical environment of the fluorophore and the electronic structure. The Stokes shift is fundamental to the sensitivity of fluorescence techniques as it allows the emission light to be separated from the excitation light. The common factors which affect the fluorescence intensity of the emission spectrum are temperature, solvent effect, pH, dissolved oxygen, concentration, and the structural rigidity of the fluorophore

***Technical grounds of Autofluorescence spectrometry***

Depending on the temporal nature of excitation and detection, fluorescence measurements can be classified as steady-state were spectrometer is implemented with constant illumination and observation and time-resolved were it is performed with pulsed excitation. Generally, steady-state measurements are simpler than time-resolved since they require less complex instrumentation and are easier for interpretation. Fluorescence measurements are of Biological materials are complex and comprise many different fluorophores, chromophores -these are molecules that absorb light but do not emit one, and light scatters. Because of that, the measurement of a single spectra, either emission or excitation or synchronous, is sometimes insufficient for the analyses and diagnostics. Excitationemission matrices (EEMs), also termed excitation-emission landscapes, are the most widely used type of multidimensional measurements They combine in a three

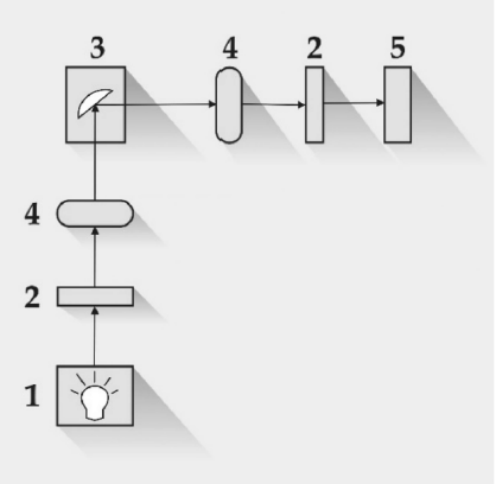
dimensional (3D) space the set of emission spectra excited by the light of different wavelengths. This EEM of a sample is its characteristic fluorescent fingerprint of the sample.

For medical diagnostics, measurements of quantum yield, polarization, and excited state lifetime may also be valuable. Quantum yield is the ratio of the number of photons emitted from fluorophore to the number of photons absorbed. Polarization gives information on the movement of fluorophore, if there is any, during the time between the absorption and emission of light, namely during the excited state lifetime.

***Laser induced fluorescence***:

The basic components in LIAF include a (1) light source, (2) wavelength selector elements on the excitation and emission paths to/from sample, (3) sample holder/positioner, (4) polarizers, and (5) detector.

FIGURE 12




Polychromatic light from light source is dispersed on a dispersing element from which light beam of selected wavelength is directed on a sample to excite fluorescence. Here Xe arc lamps were used. They produce intense monochromatic light. The single-channeled

detector, usually photomultiplier tube (PMT) or semiconductor, detects the intensity of one wavelength at a time. Multi-channeled detectors, such as charge-coupled device cameras (CCDs), record the intensity of emission over the range of wavelengths simultaneously.

## MATERIALS AND METHODS

The study was conducted after getting approval from the Institutional Ethical Committee.

**STUDY CENTRE:**

1. Department of Oral Medicine and Radiology,
   Tamil Nadu Government Dental College and Hospital,
   Chennai – 600 003.
2. Department of Radiation Oncology
   Rajiv Gandhi Government Hospital,
   Chennai -600001.
3. Department of medical physics,
   Anna University
   Guindy Chennai- 600025

**CASE SELECTION:**

Autofluorescence spectra of saliva were collected from 75 patients with who were diagnosed as OSMF, Leukoplakia, Oral Squamous Cell Carcinoma from the Department of Oral Medicine and Radiology of Tamil Nadu Government Dental College and Hospital, Chennai -600003, and Department Of Radiation Oncology, Rajiv Gandhi Government, General Hospital , Chennai-01 and 25 healthy volunteers with no clinically observable lesions were recruited to participate after they had given their informed consent. This study was approved by ethical committee of the Tamil Nadu Government Dental College and Hospital. Both the genders were included and age group of 20-70yrs were considered for the native fluorescence spectroscopic analysis of saliva The following parameters were used in the establishment of the

diagnosis: The lesions were visually inspected and palpated in the head, neck, oral, and pharyngeal regions. The procedure involved digital palpation of neck node regions, bimanual palpation of the floor of mouth and tongue, and inspection with palpation and observation of the oral and pharyngeal mucosa with an adequate light source, and mouth mirrors were used for the examination. The social, familial, and medical history was documented with risk behaviors (tobacco chewing, betel quid chewing, smoking and alcoho usage), a history of head and neck radiotherapy, familial history of head and neck cancer, and a personal history of cancer was taken and recorded and patients were reassured and counseling was given along with advice to quit their habit if present... Then the patients were selected who satisfying the following inclusion and exclusion criteria. Salivary samples from all the selected patients were collected in a sterile container, transported in ice packs within 6 hours to analyse the samples.

They are divided into 4groups

- Group I- potentially malignant lesions.
- Group II- malignant
- Group III-patients under radiotherapy (2-4weeks)
- Group IV- healthy volunteers.

***Inclusion Criteria***

- Patient with age group above the age of 20 years, both Gender are included..
- Potentially malignant conditions

  Oral Leukoplakia

  Oral submucous fibrosis.
- Oral squamous cell carcinoma

  Histological criteria of

1. Carcinoma in situ
2. Well differentiated squamous cell carcinoma
3. Moderately differentiated squamous cell carcinoma
4. Poorly differentiated squamous cell carcinoma

- Patient treated by radiotherapy
- Healthy volunteers- both genders, age above 20yrs, good oral hygiene, free from systemic illness, No habits of any form of tobacco or betel nut ad leaves use.

***Exclusion Criteria***:

- Patients with chronic systemic diseases like cardiac, cerebrovascular, Respiratory, Renal , Hepatic, Gastrointestinal complications, collagen disorders, infectious diseases, bleeding disorders, periodontal diseases.
- Pregnancy and lactation
- Patients not willing to participate in the study.

**METHODOLOGY:**

Informed consent is to be obtained from each patient prior to the investigation. Cases are to be selected from the department of oral medicine and radiology. Diagnosis of premalignant and malignant lesions are made based on clinical and histopathalogical criteria.

Incisional Biopsy was done in unconfirmed cases and subjected to histopathological study.(figure 9A, 9B )

**Armamentarium:**

**Examination of the patient: (figure 1)**

- Electrically operated dental chair
- Patient's apron
- Disposable mouth mask
- A pair of disposable latex examination gloves
- Stainless steel kidney trays
- Mouth mirror
- Stainless steel probe
- Tweezer
- Vernier calipers Metallic scale
- Sterile Saliva collecting sealed cups
- Biopsy kit **(figure 2)**
    - No 15 BP blade
    - Tissue holding forceps
    - Alleys forceps
    - Non toothed tissue holding forceps
    - Suture needle- cutting round bodied needle
    - Hemostat –curved and straight
    - Needle holder -6" inch
    - Mosquito forceps
    - Surgical gloves

***Procedure :***

Informed consent is obtained from patients. Patient draped and painted with povidine iodine under Local anaesthesia by 2% lignocaine a small wedge shaped incision( figure 9a, 9b) is made at the advancing front of the lesion and excised by a scalpel. Biopsied tissue is subject to hematoxylin and Eosin staining.

***Saliva collection***

1. Unstimulated saliva is collected from each patient. Patient is asked not to take any food 1 hour prior and rinse his mouth thoroughly with water. Patient is advised to collect saliva for 10 minutes and to collect it as it drools into the sterile container.patient is advised not to forecefully spit .(figure 5)
2. Saliva collected is transported under frozen ice within 3 hrs to department of medical physics, Anna university ,and subjected to autofluorescence spectroscopic analysis, and signatures are noted in steady state and exited emission spectrum.(figure 4, 5)

**METHOD** :

***Autofluorescence spectrometer analysis -Spectrofluorometer (Fluromax-2)***

After confirming the diagnosis the 5 ml of saliva collected from the patients are subjected to Laser induced Autofluorescence. The autofluorescence spectroscopic characterization of whole saliva were carried out using spectroflurometer of model Fluoromax-2, SPEX, Edison, New Jersey, USA. The excitation source, 150W Ozone free Xenon arc lamp coupled to the monochromator, delivers light to the sample at a desired wavelength and the fluorescence emission from the sample was collected by an emission monochromator connected

to a photomultiplier tube (R928P;Hamamatzu, Shizuoka-Ken, Japan). The gratings of the excitation and emission monochromators had a groove density of 1200 grooves $mm^{-1}$ and were blazed at 330 nm and 500 nm, respectively. Excitation and emission slit width were fixed as 5 nm. The acquisition interval and the integration time were maintained as 1 nm and 0.1 s respectively. All Fluromax – 2 functions were controlled by the DataMax software which communicates between a PC-compatible computer and the Fluromax-2 [Figures 3, Figure 4]. The DataMax software enables to specify the experimental parameters, acquire and display data, manage files, process data, specify the hardware components, control the spectrometers and supply high voltage to the signal detectors.

The steady state fluorescence emission spectra were measured in the emission range 425–750 nm at 405 nm excitation. The fluorescence excitation spectra for emission at 620 nm for the excitation range 250–600 nm were also measured.

Based on the spectrum obtained analysis of, group-I potentially malignant, group -II malignant were compared with spectrum of control group-IV and difference in the spectral intensity of specific tumour markers viz, NADH, FAD and Protoporphyrin IX (PPIX) were observed. Spectrum of Group IV were compared with group III patient under radiotherapy and difference in the spectral intensity was observed for prognostic evaluation of treated oral carcinoma patients.

**FIGURE 1 ARMEMENTARIUM FOR DIAGNOSIS**


**FIGURE 2 ARMEMNETARIUM FOR BIOPSY**

F

**IGURE 3 : FLUROMAX -2 AUTOFLUORESCENCE SPECTROMETER**


**FIGURE 4: PROJECTION UNIT**

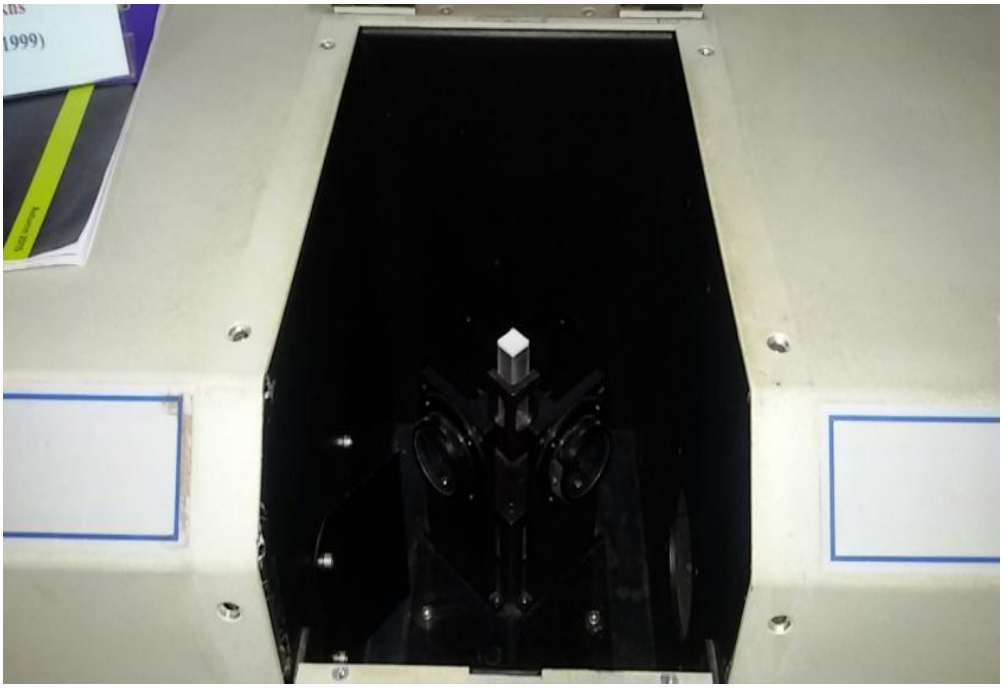


**FIGURE 5: SALIVA COLLECTION- SPIT METHOD**

**FIGURE 6 : RADIOTHERAPY – COBALT 60**

## FIGURE 7 :POTENTIALLY MALIGNANT LESIONS

### HOMOGENOUS LEUKOPLAKIA

### VERRUCOUS LEUKOPLAKIA OF TONGUE AND BUCCAL MUCOSA

### SPECKLED LEUKOPLAKIA

**FIGURE 8: ORAL SUBMUCOUS FIBROSIS**

**FIGURE : 9A ORAL SUAMOUS CELL CARCINOMA (SEEN AS MALIGNANT GROWTH CLINICALLY IN LOWER LABIAL MUCOSA)**

**FIGURE : 9B ORAL SUAMOUS CELL CARCINOMA (SEEN AS MALIGNANT GROWTH CLINICALLY IN LEFT BUCCAL MUCOSA**

**FIGURE : 9C MALIGNANT ULCER OF TONGUE CLINICALLY BENIGN MALIGNANT LESION**

**FIGURE 10A :INCISIONAL BIOPSY OF VERRUCOUS LEUKOPLAKIA IN RIGHT BUCCAL MUCOSA**

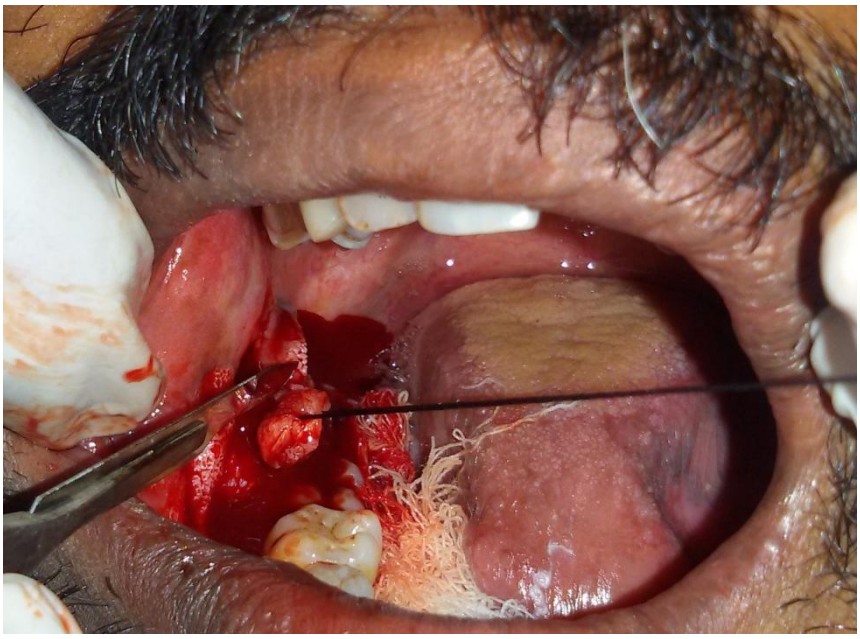
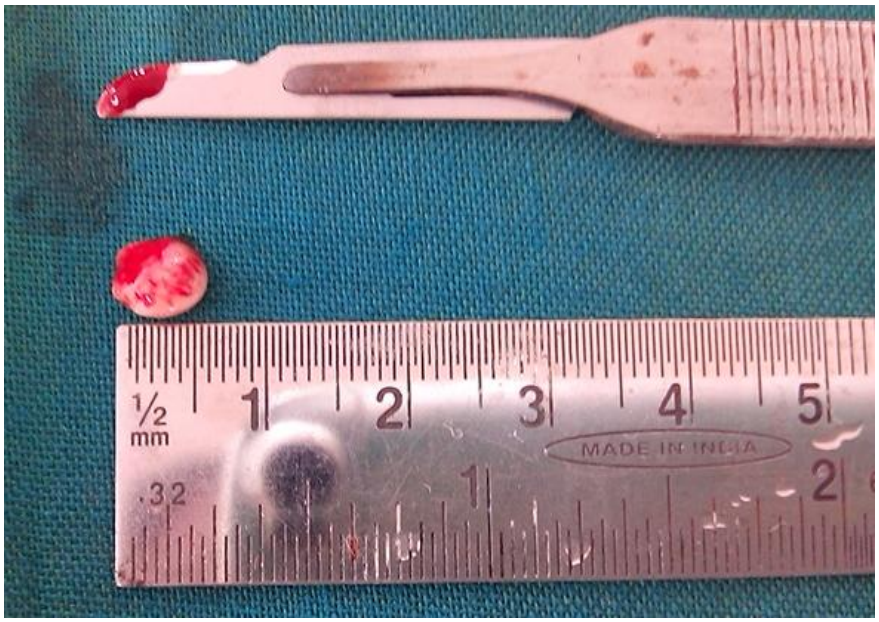


**FIGURE 10B**



**PATIENTS UNDER RADIOTHERAPY**

**FIGURE 11A: AFTER 2ND WEEK OF RADIATION**

**FIGURE 11 B: AFTER 3RD WEEK OF RADIATION**

## RESULTS

A total of 100 saliva samples from patients who fulfilled the inclusion and exclusion criteria were selected for the study. The study groups were equally divided into four groups, they are, group I- oral potentially malignant disorder patient's saliva, group-II oral squamous cell carcinoma patient's saliva, group –III radiotherapy patient's saliva and group-IV –normal saliva. The age range for group-I was 20- 62 years with a mean age of 46 years. The age range for group-II was 45-66years with a mean age of 56years. The age range for group-III was 22yrs- 56yrs with a mean age of 46.60years. The age range for group-IV was 19 to 80 years with a mean age of 36.08years.(table II, chart 5 )

The group-I patients included 18 males (72%) and 7 females(28%). Out of the 25 patients, 10 patients had OSMF, 15 patients had oral leukoplakia and 1 patient had both OSMF and leukoplakia. About 11 patients had leukoplakia on buccal mucosa, of which 6 had on right buccal mucosa and 5 had on left buccal mucosa. About 5 patient had leukoplakia on the tongue with 1 patient over dorsum, 2 patient on left lateral border and 3 on right lateral border. 6 patients had OSMF involving the buccal mucosa, and soft palate bilaterally and 4 patients had involvement of buccal mucosa alone.(chart 4)

The group-II patients were 12 males(48%) and 13 females(52%), of which 11(44%) patient had lesion involving buccal mucosa, 7(28%) patients had lesions in tongue, and 2(8%) patient had lesion on palate, and 5(20%) patients had lesion involving gingivobuccal complex (chart 3). Based on TNM staging about 24% of group II patients were in T4N2bM0, and T3N0M0. 16% patients were in T2N0M0 and T3N2aM0 stage, 4% of patients were in T4N0M0and T2N2aM0 staging.

The group-III patients were 15(60%) males and 10(40%)females, of which 10 patients were in $2^{nd}$ week of radiotherapy 7 patients were in $3^{rd}$ week of radiotherapy and 7 patients were in $4^{th}$ week of radiotherapy.

The group IV patients were 14(56%) males and 11(44%) females. (table II ) About 5ml of unstimulated whole saliva collected from all four groups were subjected to laser induced autofluorescence excitation in the region of visible spectrum at 405nm as steady state excitation and at 620nm steady state excitation the Spectrum thus obtained were measured and compared for all groups.

***Fluorescence emission spectra for 405 nm excitation spectrum( graphs1a )***

The fluorescence emission spectral characteristics of group-I saliva, group-II saliva, group-III saliva, and group-IV saliva were measured for 405 nm excitation. The saliva was excited at 405nm to assess the spectral characteristics of the tumour maker protoporphyrin (PPIX) in the first three groups and were compared with the spectrum of normal saliva.

The spectrum of group IV showed two prominent peaks one at 467nm at a fluorescence intensity of $4.5 \times 10^5$ and second peak at 625nm with an intensity of $2 \times 10^5$ .

The spectrum of group I showed two prominent peaks one at 467nm at a fluorescence intensity of $5.25 \times 10^5$ and second peak at 625nm with an intensity of $2.25 \times 10^5$ showing a spectral difference of 1.125 times between group I and IV at 625nm.

The spectrum of group II showed two prominent peaks one at 467nm at a fluorescence intensity of $5.25 \times 10^5$ and second peak at 625nm with an intensity of $4 \times 10^5$ showing a two fold rise in the spectral intensity between group IV and group II at 625nm.

The spectrum of group III showed two prominent peaks one at 467nm at a fluorescence intensity of $5.25 \times 10^5$ and second peak at 625nm with an intensity of $2.25 \times 10^5$ showing a spectral difference of 1.125 times between group I and III at 625nm.

The peaks at 625nm in the spectrum shows elevation of protoporphyrin level at 625nm for group I, II, and III patients when compared to spectrum of group IV patient's saliva. The fluorescence intensity of group II and group III is being similar at 625nm region

shows a decrease in porphyrin level in group III saliva compared to the spectrum of goup II saliva. The peaks around 467nm region corresponds to the lamp peak of the Xenon arc of Fluoromax-2 arising as result of stray light from the source

**Normalized Average Fluorescence emission spectra at 405 nm excitation: (graph 1b )**

The normalized average fluorescence emission spectrum group IV saliva showed a smooth peak at 625nm with an intensity of 0.2 a.u (arbitrary unit)

The normalized average fluorescence emission spectrum group I showed a shallow peak at 625nm with an intensity of 0.3 a.u .

The normalized average fluorescence emission spectrum group II showed a prominent peak at 625nm with an intensity of 0.8 a.u showing a 4times rise in the spectral intensity between group IV and group II saliva.

The normalized average fluorescence emission spectrum group III showed a prominent peak at 625nm with an intensity of 0.4 a.u showing a 2 times rise in the spectral intensity between group IV and group II saliva.

The normalized average fluorescence emission spectrum of OSCC saliva showed a peak at 460 nm corresponding to lamp peak The normalized average fluorescence emission spectrum at 405nm had a primary band centered around 460 nm and then it decreased towards longer wavelengths and takes a peak at 625 nm in all four groups.

**DIFFERENCE SPECTRUM:**

The normalised spectra values between group IV and group I was plotted for difference in intensity of the spectrum. The difference spectrum was computed by subtracting the normalized average fluorescence emission spectrum of group IV from that of group I, group II and group III saliva spectrum separately. The difference thus measured were,

a. About 0.2 au between group I and group IV(**graph 2a**).
b. About 0.6 au between group II and group IV(**graph 2b** ).

c. About 0.25 au between group III and group IV**(graph 2c**).

The inverse peak at 450-475nm band corresponding to NADH with an intensity of 0.375 a.u was seen after exclusion of the lamp peaks at 467nm, and this intensity was found to be equal in all three groups showing no changes in levels of NADH among the saliva of he four groups.

**Fluorescence Excitation Spectral Characteristics Of Saliva At 620nm :(graph 3a )**

In order to cross validate whether the emission peaks at 625 nm were due to porphyrin, the fluorescence excitation spectra of group I, group II, group III, group IV patient's saliva were measured for emission at 620 nm.

The spectrum of group IV showed two prominent peaks one at 405nm at a fluorescence intensity of 1.8 x $10^5$ cps and second smooth peak at 510nm corresponds to levels of flavins (FAD) showed an intensity of 0.8 x $10^5$ Cps.

The spectrum of group I showed two prominent peaks one at 405 nm at a fluorescence intensity of 1.825 x $10^5$ cps and second peak at 510 nm corresponds to levels of flavins (FAD) showed an intensity of 0.8 x $10^5$ cps showing a minimal spectral difference of between group I and IV at 405 and 510nm

The spectrum of group II showed two prominent peaks one at 405 nm at a fluorescence intensity of 3.30 x $10^5$ cps and second peak at 510 nm corresponds to levels of flavins (FAD) showed an intensity of 1.10 x $10^5$ cps showing a spectral difference of 1.83 times between group II and IV at 405nm and 1.37 between group II and IV.

The spectrum of group III showed two prominent peaks one at 405 nm at a fluorescence intensity of 2.475 x $10^5$ cps and second peak at 510 nm corresponds to levels of flavins (FAD) showed an intensity of 1 x $10^5$ cps showing a spectral difference of 1.37 at 405nm and 1.25 times higher spectral difference at 510nm between group III and IV

The peak at 405 nm in the normalized average fluorescence excitation spectrum of group I, group II, group III, group IV, saliva may be attributed to the Q-band absorption of endogenous porphyrin. The normalisation is done at 620nm excitation to analyse the shift in the spectrum and there was considerable shift in the spectral line between group I, group II, group III, group IV saliva 405nm, and 510nm showing alterations in levels of porphyrin and FAD of group I,II, and III saliva from Group IV saliva.

**DIFFERENCE SPECTRUM: (graph 4)**

Excitation difference spectrum at 620nm was plotted for comparing the difference in spectral intensity between group IV saliva from group I, group II, group III saliva. The difference spectrum was computed by subtracting the normalized average fluorescence emission spectrum of group IV from that of group I, group II and group III saliva spectrum separately. The difference thus measured were,

a. About 0.05 au between group I and group IV at 405nm and 0.1au at 510nm **(graph 4a).**
b. About 0.125 au between group II and group IV at 405nm and 0.05au at 510nm **(graph 4b ).**
c. About 0.1 au between group III and group IV at 405nm and 0.1au at 510nm **(graph 4c).**

**Table 1**

## ENDOGENOUS FLUOROPHORES

| FLUROPHORES | DESCRIPTION | EXCITATION MAXIMA | EMMISSION MAXIMA |
|---|---|---|---|
| NADH | CO-ENZYME | 290,340, 350,365 | 440,450,455,460 |
| NADPH | CO-ENZYME | 336 | 464 |
| FAD,FLAVINS | CO-ENZYME | 450 | 515,535 |
| COLLAGEN | STRUCTURAL PROTIENS | 270, 325,330,335 | 380,390-405, 395, 400,405 |
| COLLAGEN CROSS LINKS | STRUCTURAL PROTIENS | 370 | 460-490 |
| ELASTIN | STRUCTURAL PROTIENS | 290, 325,350,360 | 340, 400, 420, 460 |
| ELASTIN CROSS LINKS | STRUCTURAL PROTIENS | 390-420,400, 420-460 | 500, 500-540 |
| TRYPTOPHAN | AMINOACIDS | 280, 295 | 350, 340-350, 345 |
| TYROSINE | AMINOACIDS | 275 | 300,340 |
| PHENLALANINE | AMINOACIDS | 260 | 280 |
| PHOSPHOLIPIDS | LIPIDS | 436 | 540, 560 |
| CEROID | LIPIDS | 340-395 | 430-460, 540 |
| KERATIN | FIBROUS PROTIEN | 365,370, 375 | 430, 460 |
| PYRIDOXINE | VITAMIN | 332 | 400 |
| PORPHYRINS | PORPHYRIN | 405,400-450, 630 | 635, 690, 705 |
| LIPOFUSCIN | PIGMENT | 340-395 | 430-460, 540 |
| EOSINOPHILS | BLOOD CELLS | 370, 500 | 440, 550 |

**TABLE II:**

**AGE AND GENDER DISTRIBUTION AMONG THE FOUR GROUPS**

| Group | Number of Patients | Male | female | Age group years | Mean age years |
|---|---|---|---|---|---|
| Group I | 25 | 18(72%) | 7(18) | 20-62 | 46 |
| Group II | 25 | 12 (48%) | 13(52%) | 45-66 | 56 |
| Group III | 25 | 15(60%) | 10(40%) | 22-56 | 47 |
| Group IV | 25 | 14(56%) | 11(44%) | 19-80 | 36 |

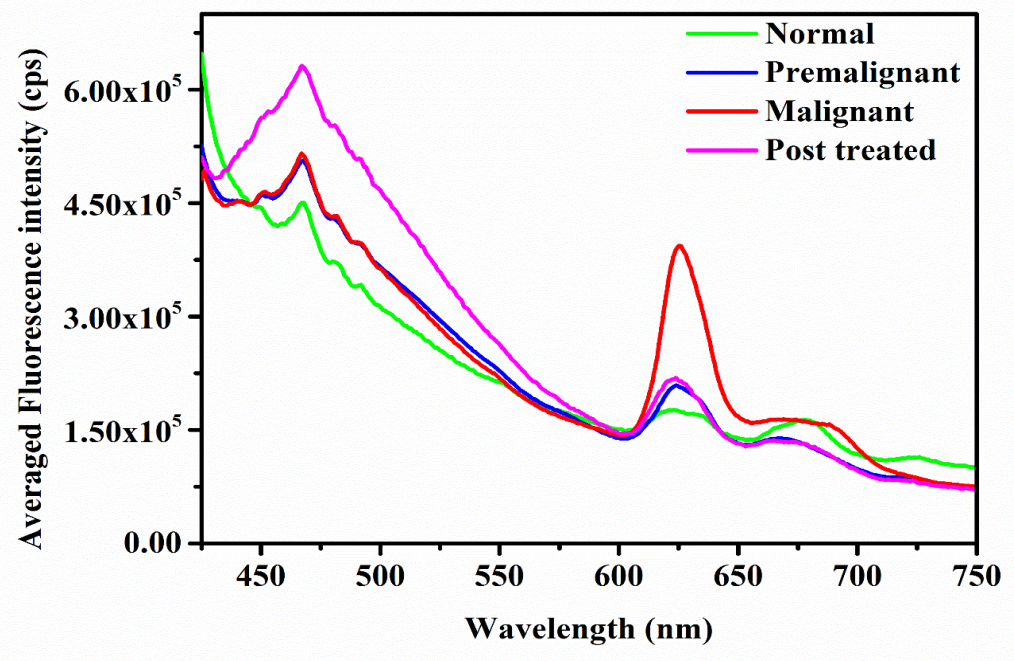
**TABLE 3 : PCA- LDA ANALYSIS FOR 405nm EMISSION SPECTRA**

| SPECTRA | SENSITIVITY | SPECIFICITY | P-VALUE |
|---|---|---|---|
| **Fluoresce emission for group I at 405nm** | **76.0** | **42.9** | **<.001** |
| **Fluoresce emission for group II at 405nm** | **73.9** | **42.9** | **<.001** |
| **Fluoresce emission for goup III at 405nm** | **80.0** | **42.9** | **<.001** |

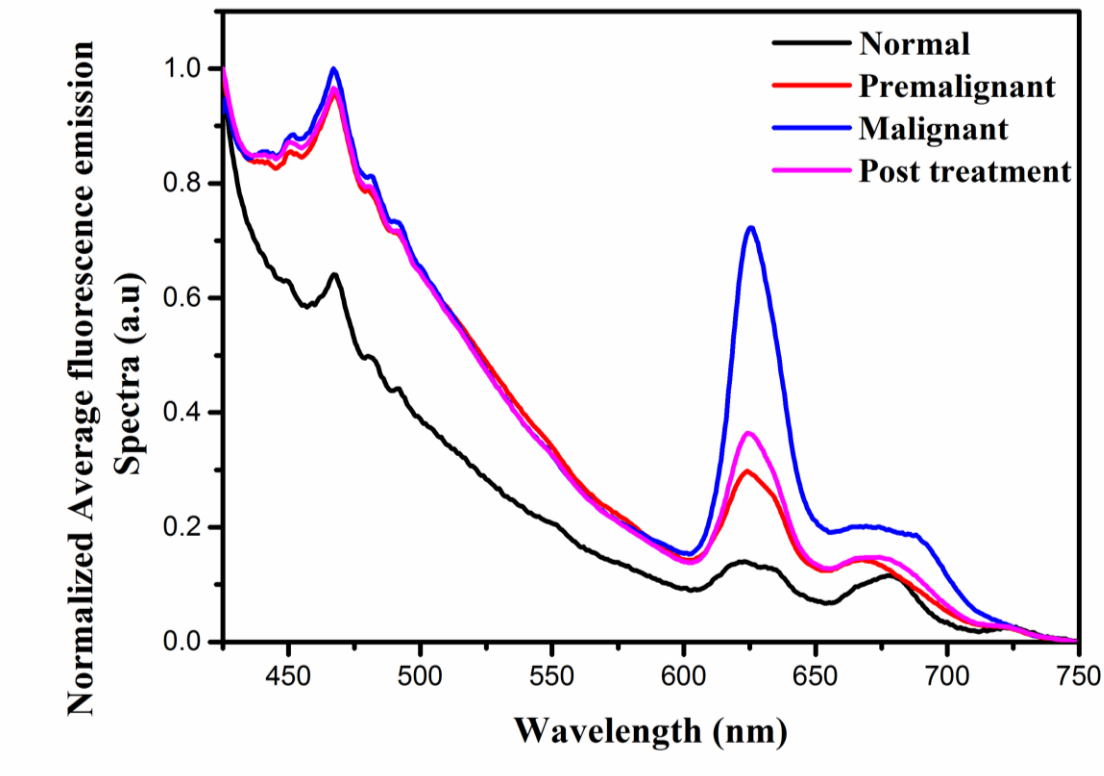
**TABLE 4 : PCA- LDA ANALYSIS FOR 620nm EMISSION SPECTRA**

| SPECTRA | SENSITIVITY | SPECIFICITY | P-VALUE |
|---|---|---|---|
| **Fluoresce emission for group I at 620nm** | **75.0** | **42.9** | **<.001** |
| **Fluoresce emission for group II at 620nm** | **85.7** | **52.2** | **<.001** |
| **Fluoresce emission for group III at 620nm** | **85.7** | **60.0** | **<.001** |

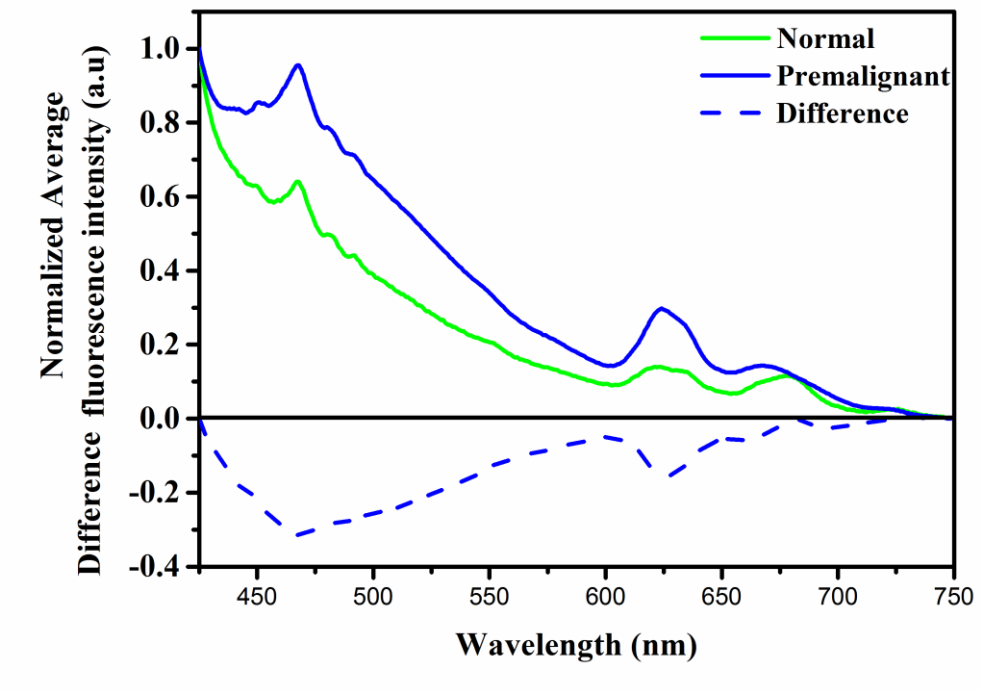
**GRAPH 1a: 405 nm excitation emission spectra**



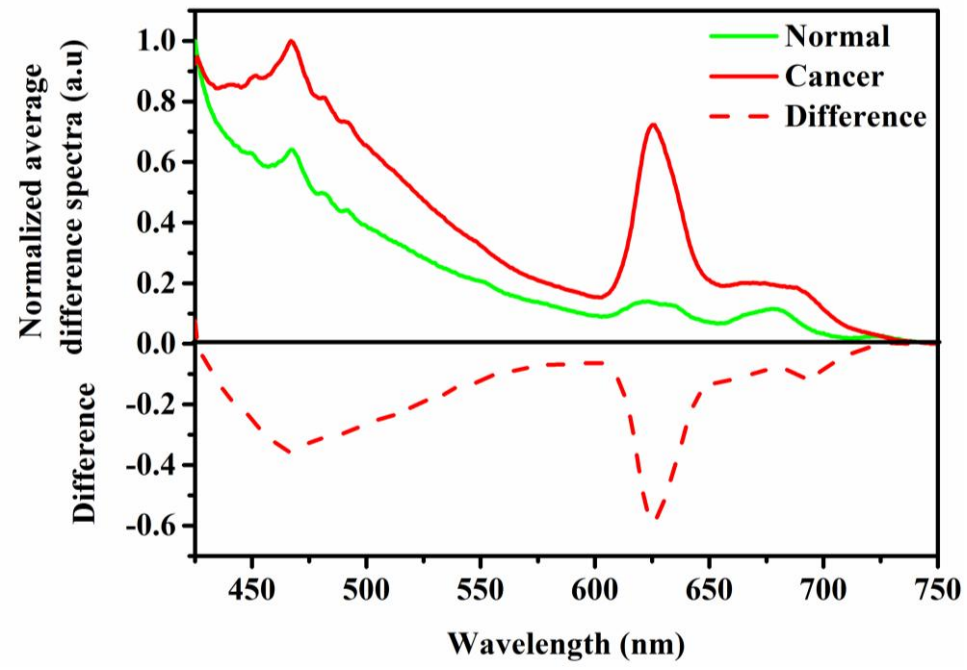

**GRAPH 1b: Normalized Average Fluorescence emission spectra at 405 nm excitation**



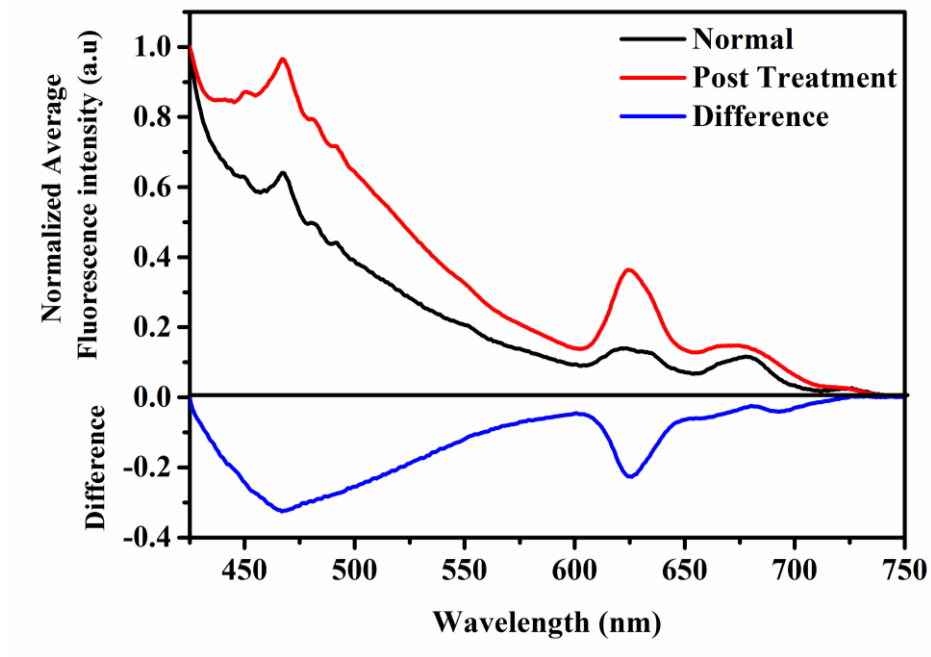
**GRAPH 2a : Normalized Average Fluorescence emission spectra of normal and oral premalignant lesion at 405 nm excitation and its difference**



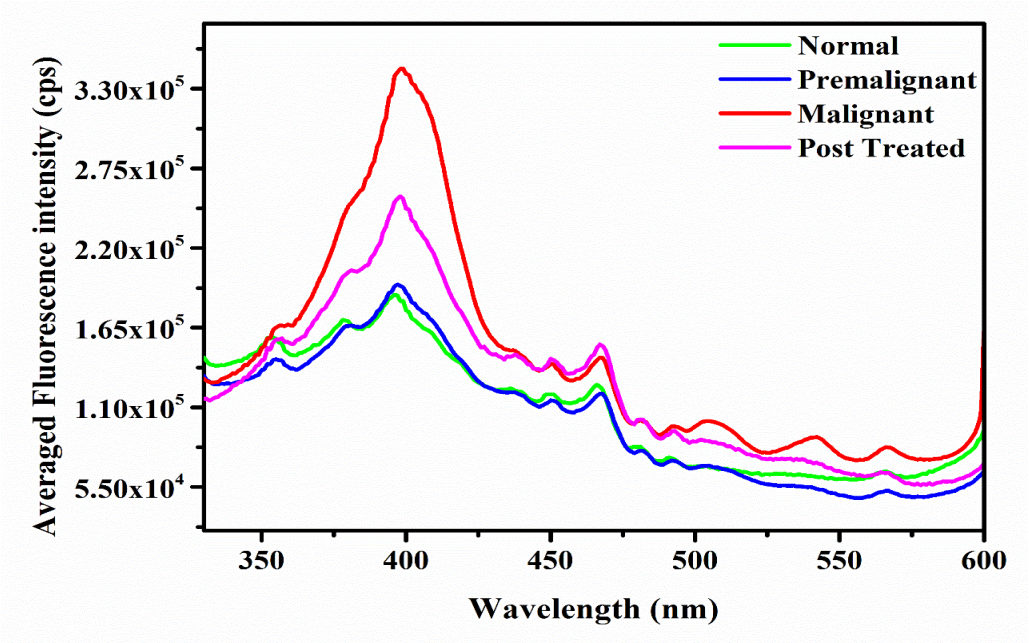

**GRAPH 2b: Normalized Average Fluorescence emission spectra of normal and oral malignant patients at 405 nm excitation and its difference**



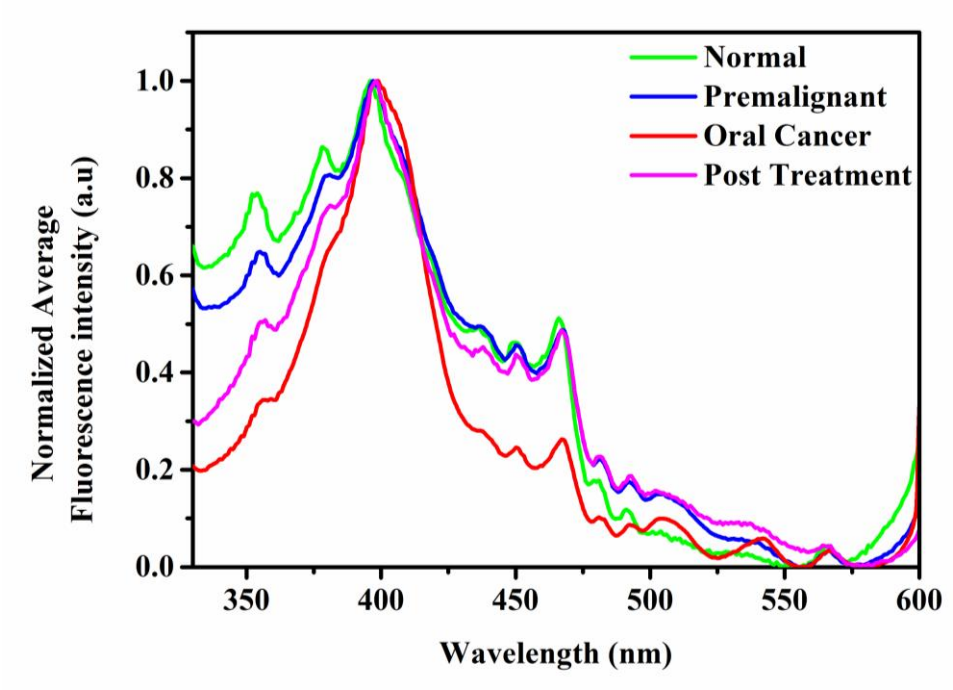
**GRAPH 2c :Normalized Average Fluorescence emission spectra of normal and Post treatment at 405 nm excitation and its difference**



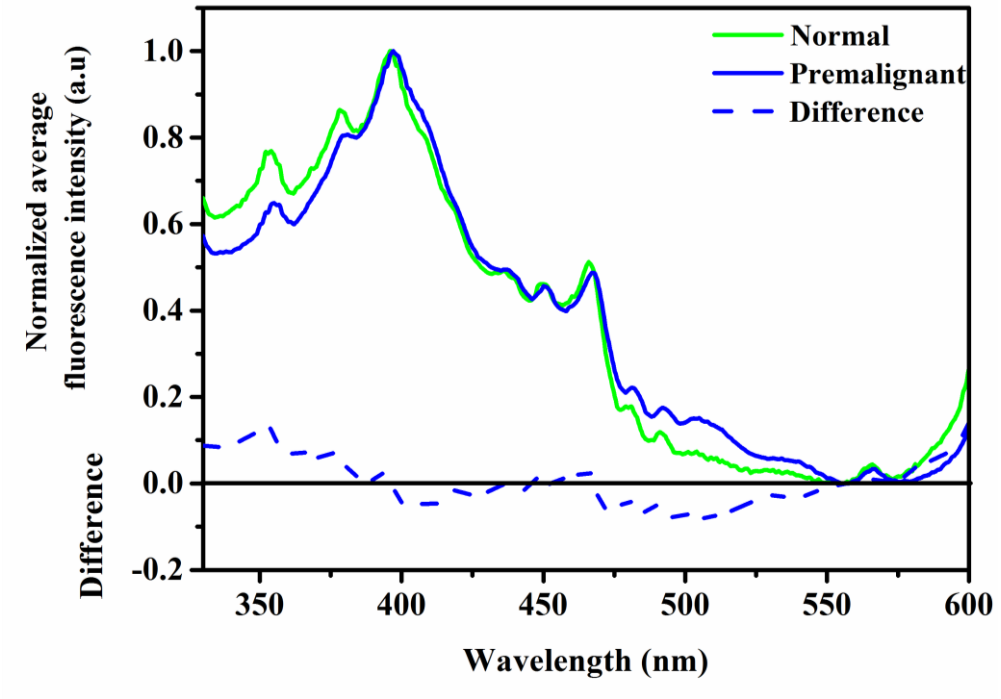

**GRAPH 3a :620 nm emission maximum excitation spectra**



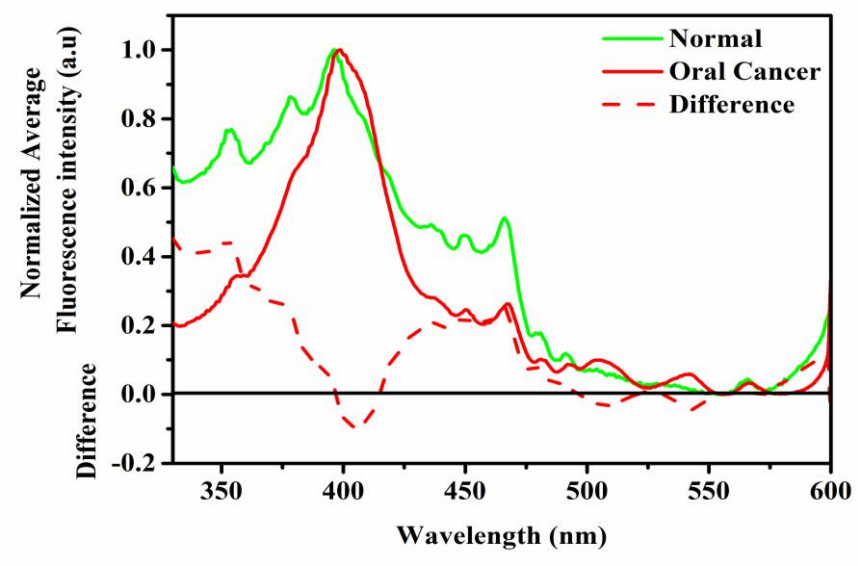
**GRAPH 3b: Normalized Average Fluorescence excitation spectra for 620 nm emission maximum**



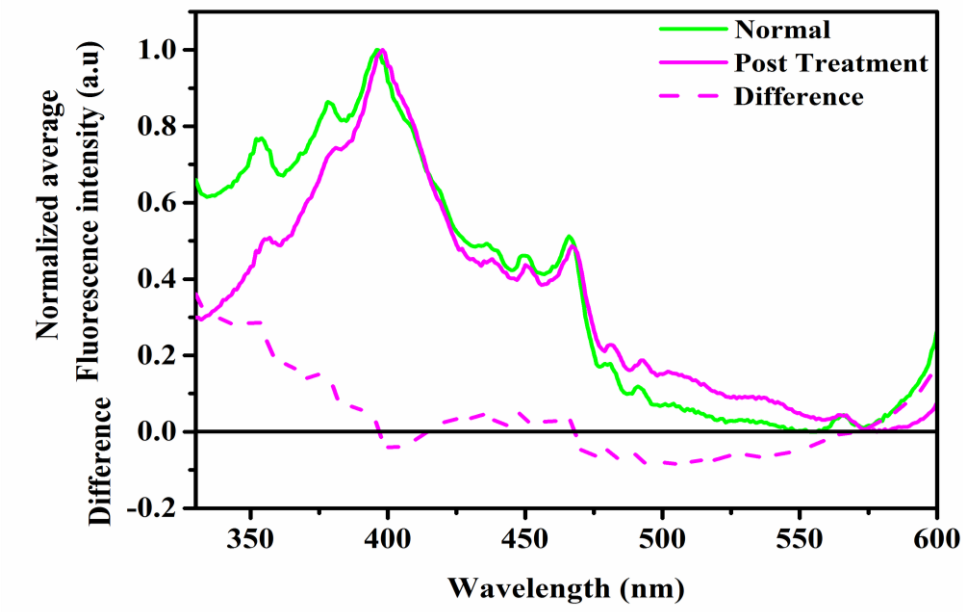

**GRAPH 4a :Normalized Average Fluorescence excitation spectra for 620 nm emission maximum of normal and oral premalignant and its difference spectra**



**GRAPH 4b : Normalized Average Fluorescence excitation spectra for 620 nm emission maximum of normal and oral malignant and its difference spectra**




**GRAPH 4c :Normalized Average Fluorescence excitation spectra for 620 nm emission maximum of normal and Post Treatment and its difference spectra**



**Statistical analysis:**

Statistical analysis of specificity, sensitivity of the study was done by IBM SPSS version 19.0

Principal component analysis (PCA) was done for extraction of spectral internsity data and further analyzed and data discrimination was done by linear discriminant analysis (LDA)

**Table 5 :PCA – LDA of normal vs cancer**

**Classification Results[a,c]**

| | | VAR00033 | Predicted Group Membership | | Total |
|---|---|---|---|---|---|
| | | | 1.00 | 2.00 | |
| Original | Count | 1.00 | 12 | 13 | 25 |
| | | 2.00 | 6 | 17 | 23 |
| | % | 1.00 | **42.9** | 57.1 | 100.0 |
| | | 2.00 | 26.1 | **73.9** | 100.0 |
| Cross-validated[b] | Count | 1.00 | 12 | 13 | 25 |
| | | 2.00 | 6 | 17 | 23 |
| | % | 1.00 | **42.9** | 57.1 | 100.0 |
| | | 2.00 | 26.1 | **73.9** | 100.0 |

a. 66.7% of original grouped cases correctly classified.

b. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

c. 66.7% of cross-validated grouped cases correctly classified

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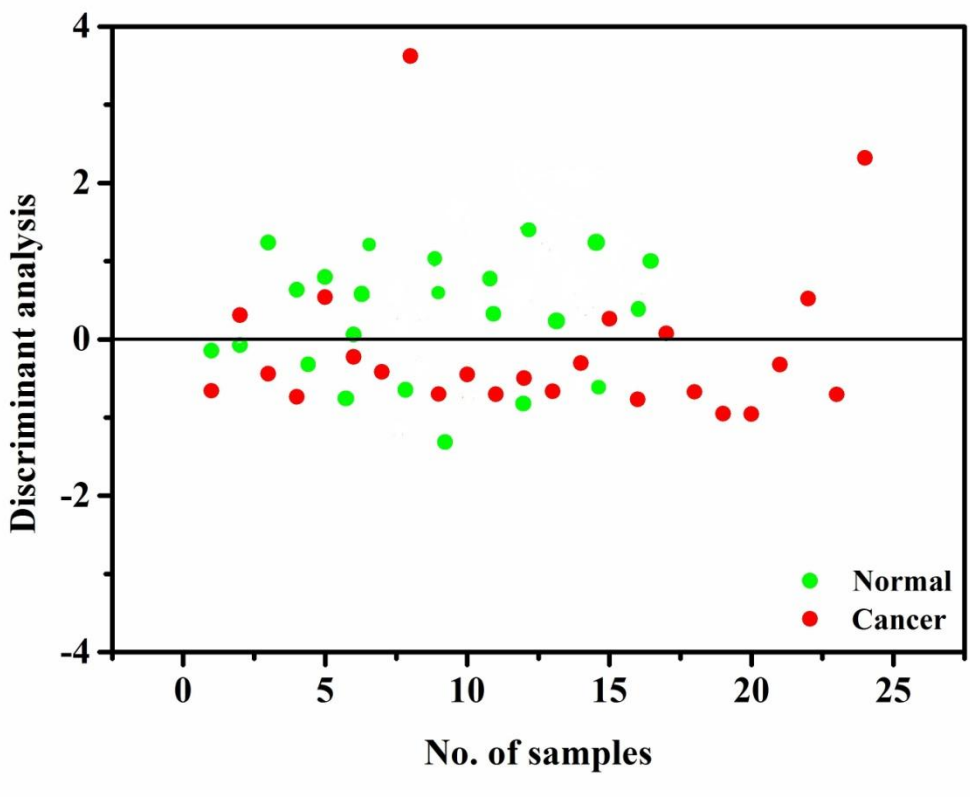



**Table 6 :PCA – LDA of normal vs Premalignant**

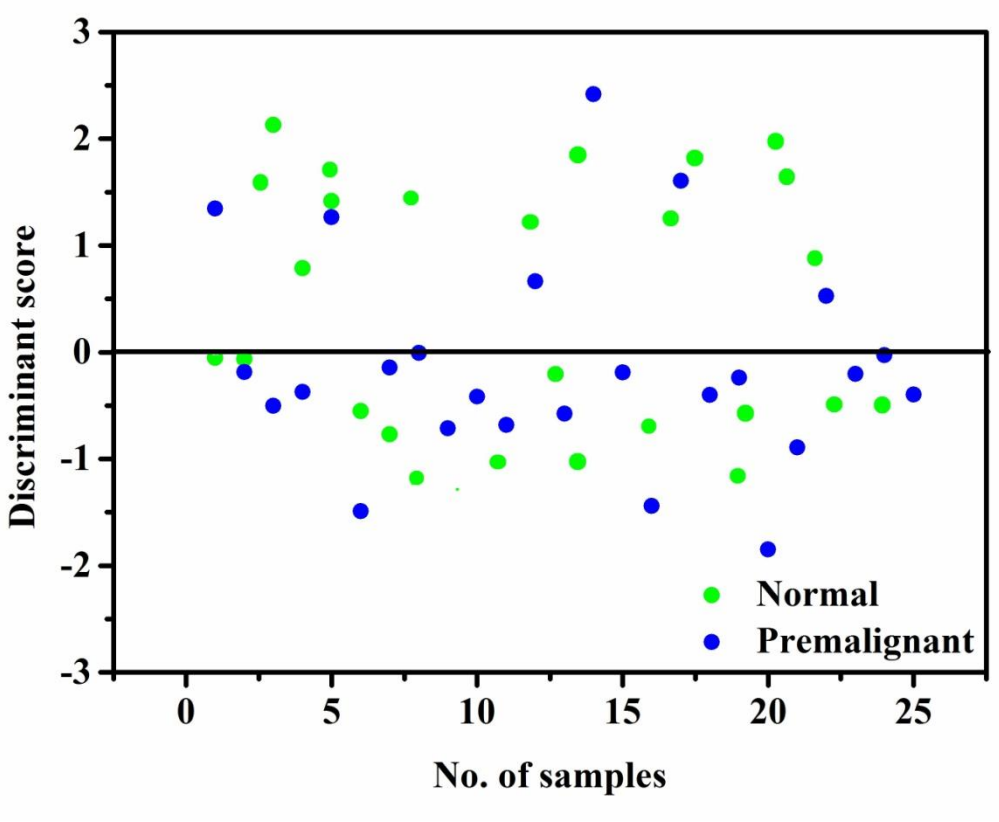
**Classification Results[a,c]**

| | | VAR00033 | Predicted Group Membership | | Total |
|---|---|---|---|---|---|
| | | | 1.00 | 2.00 | |
| Original | Count | 1.00 | 12 | 13 | 25 |
| | | 2.00 | 6 | 19 | 25 |
| | % | 1.00 | 42.9 | 57.1 | 100.0 |
| | | 2.00 | 24.0 | 76.0 | 100.0 |
| Cross-validated[b] | Count | 1.00 | 12 | 13 | 25 |
| | | 2.00 | 6 | 19 | 25 |
| | % | 1.00 | 42.9 | 57.1 | 100.0 |
| | | 2.00 | 24.0 | 76.0 | 100.0 |

a. 68.8% of original grouped cases correctly classified.

b. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

c. 68.8% of cross-validated grouped cases correctly classified.




## Table 7: PCA – LDA of normal vs Post treatment

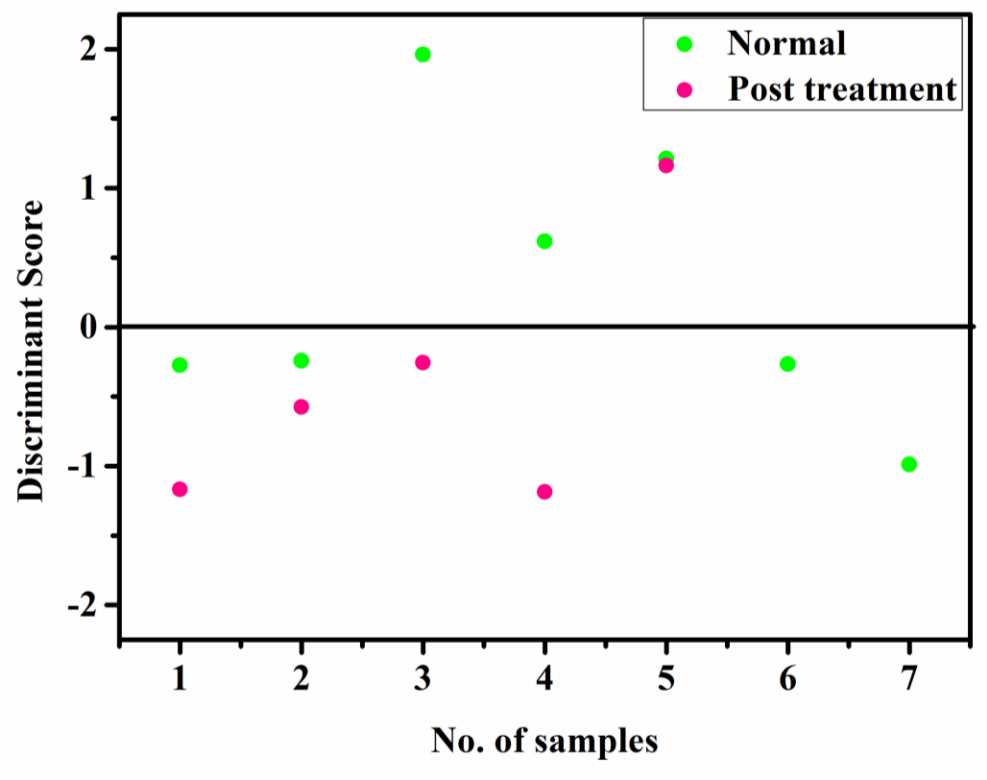
**Classification Results[a,c]**

| | | VAR00001 | Predicted Group Membership | | Total |
|---|---|---|---|---|---|
| | | | 1.00 | 2.00 | |
| Original | Count | 1.00 | 3 | 4 | 7 |
| | | 2.00 | 1 | 4 | 5 |
| | % | 1.00 | **42.9** | 57.1 | 100.0 |
| | | 2.00 | 20.0 | **80.0** | 100.0 |
| Cross-validated[b] | Count | 1.00 | 3 | 4 | 7 |
| | | 2.00 | 1 | 4 | 5 |
| | % | 1.00 | **42.9** | 57.1 | 100.0 |
| | | 2.00 | 20.0 | **80.0** | 100.0 |

a. 58.3% of original grouped cases correctly classified.

b. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

c. 58.3% of cross-validated grouped cases correctly classified.




## Table 8 :PCA – LDA of normal vs Premalignant

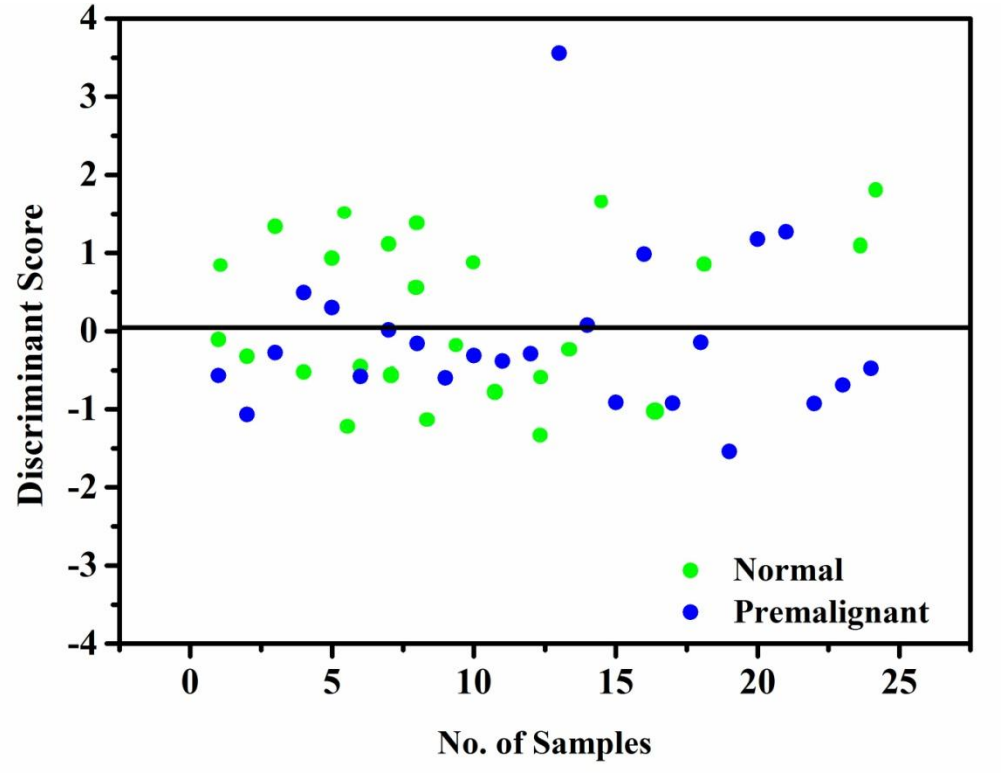
**Classification Results[a,c]**

| | | VAR00001 | Predicted Group Membership | | Total |
|---|---|---|---|---|---|
| | | | 1.00 | 2.00 | |
| Original | Count | 1.00 | 12 | 13 | 25 |
| | | 2.00 | 6 | 18 | 24 |
| | % | 1.00 | **42.9** | 57.1 | 100.0 |
| | | 2.00 | 25.0 | **75.0** | 100.0 |
| Cross-validated[b] | Count | 1.00 | 12 | 13 | 25 |
| | | 2.00 | 6 | 18 | 24 |
| | % | 1.00 | **42.9** | 57.1 | 100.0 |
| | | 2.00 | 25.0 | **75.0** | 100.0 |

a. 67.7% of original grouped cases correctly classified.

b. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

c. 67.7% of cross-validated grouped cases correctly classified.




## Table 9 :PCA – LDA of normal vs cancer

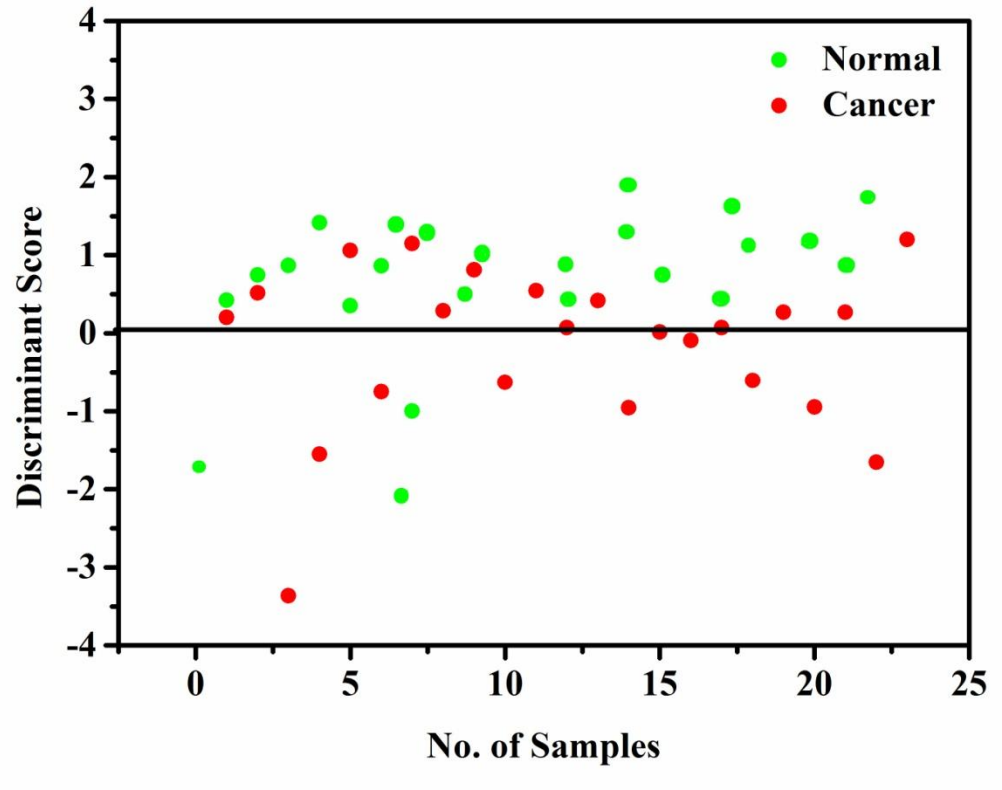
**Classification Results[a,c]**

| | | VAR00001 | Predicted Group Membership | | Total |
|---|---|---|---|---|---|
| | | | 1.00 | 2.00 | |
| Original | Count | 1.00 | 22 | 3 | 25 |
| | | 2.00 | 11 | 12 | 23 |
| | % | 1.00 | 85.7 | 14.3 | 100.0 |
| | | 2.00 | 47.8 | 52.2 | 100.0 |
| Cross-validated[b] | Count | 1.00 | 22 | 3 | 25 |
| | | 2.00 | 11 | 12 | 23 |
| | % | 1.00 | 85.7 | 14.3 | 100.0 |
| | | 2.00 | 47.8 | 52.2 | 100.0 |

a. 60.0% of original grouped cases correctly classified.

b. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

c. 60.0% of cross-validated grouped cases correctly classified.




## Table 10 :PCA – LDA of normal vs Post Treatment

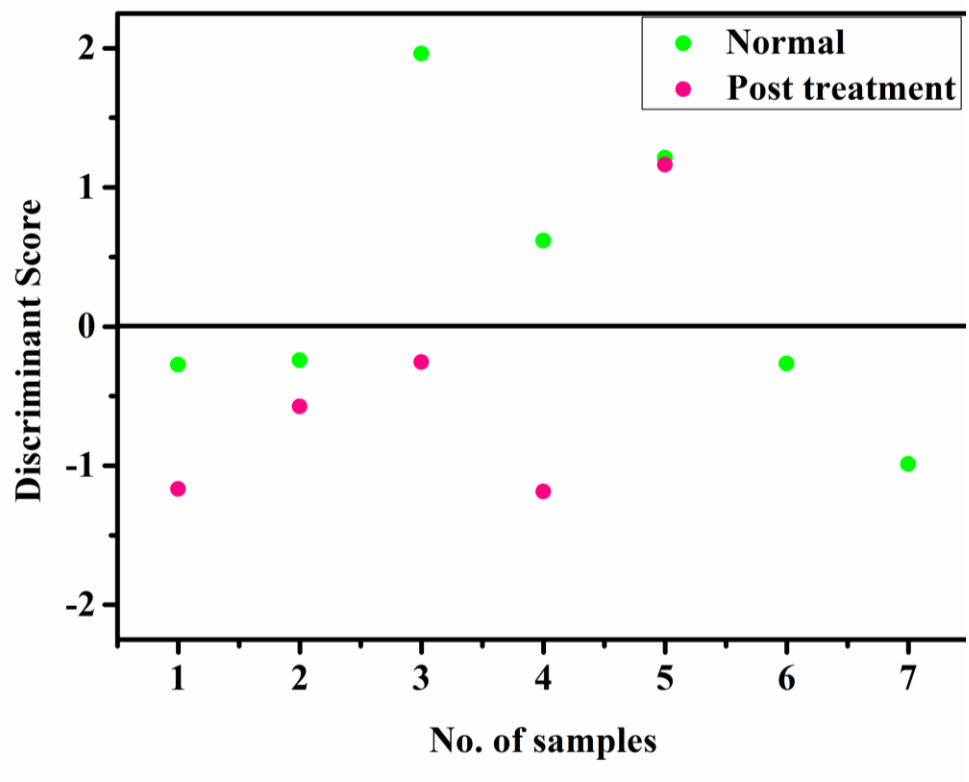
**Classification Results[a,c]**

| | | VAR00001 | Predicted Group Membership | | Total |
|---|---|---|---|---|---|
| | | | 1.00 | 2.00 | |
| Original | Count | 1.00 | 25 | 0 | 25 |
| | | 2.00 | 12 | 13 | 25 |
| | % | 1.00 | 100.0 | .0 | 100.0 |
| | | 2.00 | 40.0 | 60.0 | 100.0 |
| Cross-validated[b] | Count | 1.00 | 22 | 3 | 25 |
| | | 2.00 | 12 | 13 | 25 |
| | % | 1.00 | 85.7 | 14.3 | 100.0 |
| | | 2.00 | 40.0 | 60.0 | 100.0 |

a. 83.3% of original grouped cases correctly classified.

b. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

c. 75.0% of cross-validated grouped cases correctly classified.



## DISCUSSION

Optical spectroscopy methods have had considerable impact in the field of biomedical diagnostics, providing novel methods for the early or noninvasive diagnosis of various medical conditions. Among them, fluorescence spectroscopy has been the most widely explored mainly because fluorescence is highly sensitive to the biochemical makeup of tissues. Studies done by Jayachandran et al has shown that tumors were easily detected on account of altered fluorescence properties with respect to fluorescence of ordinary tissue[48,,84] . Even though it is easy detect oral cancer clinically as it is located in plain site with their clear visibility and easy accessibility. However, it was worth to note that the clinical examination of oral lesion may sometime mislead the investigation, many clinically benign and painless lesion at an early stages escapes detection on routine dental examination. Thus late detection of these lesions are the main reason for highest mortality rate for oral cancer. These circumstances demand the need for a reliable tumor marker(s) to evaluate the premalignant and malignant conditions and also to monitor the therapeutic efficacy.

Based on these, many tumor markers on body biofluids such as blood, urine and saliva were identified (venkatakrishna et al). Currently, much attention has been drawn on the analysis of saliva to detect various diseases.Since lesions in the oral cavity is always bathed by saliva and the exfoliated cells from dysplastic premalignant cells and exfoliated damaged Oral carcinoma cells shows significant alterations with possibility of transforming many key biomolecules such as proteins, peptides, nucleic acid, electrolytes and hormones from blood capillaries to saliva either by passive diffusion of lipophilic molecules or active transport of proteins via ligand receptor binding[4, 28].

Tatjana Dramićanin et al studied the the potential of autofluorescence spectroscopy in differentiating various malignant tissues such as oral, cervix, breast.[85] The photophysical characterizations of autofluorescence spectroscopy allows the detection of intrinsic biomolecules which were present in blood, urine, and saliva. Soukos et al has reported on the use of autofluorescence spectroscopy of saliva for forensic applications for detecting biomolecular changes in saliva of the victims. This current study the principle of Autofluorescence was used to detect the presence of intrinsic fluorophores(such as endogenous Protophyrin IX, NADH, FAD) as a potential tumor marker for early diagnosis of potentially malignant disorders, oral carcinoma and to evaluate treatment prognosis in oral carcinoma patients under radiotherapy.

In this study it was observed that the emission spectra of group I, group II, group III, group IV, exhibit a major peak around 454 to 475 nm as seen in the difference spectrum figures . This emission peak may be attributed to free and/or enzyme bound NADH/NAD(P)H. However, saliva of group I, group II, group III patients exhibit two more additional peaks centered at 625 nm and 687 nm corresponding to porphyrins (PpIX). The spectrum clearly shows elevation of protoporphyrin level at 625nm for group I, group II, group III patient's saliva when compared to spectrum of normal saliva patients. The spectral intensity of group I patients was $1.825 \times 10^5$ cps with a rise of PpIX level in group I is about 1.125 times more than group IV. This alteration in the porphyrin level signifies the metabolic changes within the dysplastic cells of potentially malignant disorders.

The spectral intensity of group I patients was $3.30 \times 10^5$ cps with a rise of PpIX level in group II is about two times more than group IV(figure 1a, 1b). This alteration in the porphyrin

level signifies the metabolic changes within the cancer cells leading to accumulation porphyrins in the exfoliated tumour cells in the saliva.

The spectral intensity of group III patients was 2.475 x $10^5$ cps with a rise of porphyrin level in group III about 1.37 times more than group IV showing the level porphyrin in patients under treatment is higher than normal patients but there was considerable reduction in porphyrin levels following treatment, thus showing the prognosis in such patients.(graph 2a,2b)

Fluorescence excitation at 620 nm showed another peak at 510nm region corresponding to flavins (FAD) with a spectral intensity of 1.10x$10^{-5}$ in group I and 0.8x$10^{-5}$ was obtained. The level of FAD in group I showed a 1.37 times the rise than group IV saliva. ( graph 3a, 3b)

The spectrum of group III patients showed FAD peak at 510nm with a spectral intensity of 1 x $10^5$ cps which was 1.25 times higher than group IV saliva thus showing the rise in FAD values in saliva of patients under treatment than normal saliva(graph 3b). However this rise is less than that seen oral cancer patients suggesting a decrease in level of FAD following treatment, as number of cancer cells exfoliated in saliva is less[86,87] following their destruction by radiation as shown in the studies done by Sreebny L et al and Silverman S jr et al.

Statistical analysis by PCA-LDA analysis showed a significant sensitivity of 76%, for 405nm excitation and 75% at 620nm excitation with a p-value < 0.01 for group I saliva. Thus the study clearly discriminates the spectral difference in potentially malignant saliva from normal saliva there by proving the efficacy of autofluorescence in detecting potentially malignant disorders.

Statistical analysis by PCA-LDA analysis showed a significant sensitivity of 73.9%, at 405nm excitation and 85.7% at 620nm excitation with a p-value < 0.01 for group II saliva. Thus

based on spectral characterization of proteins flavins and porphrins in saliva diagnosis of oral cancer at an early stage.

Statistical analysis by PCA-LDA analysis showed a significant sensitivity of 80.0%, for 405nm excitation and 85.7% at 620nm excitation with a p-value < 0.01 for group III saliva.

However PCA- LDA statistical analysis shows decreased specificity of 42.9%, 52.2%, and 60% for group I, group II and group III patient's saliva.

Dysplastic cells of potentially malignant disorder and oral cancer have altered metabolic rate either by direct or indirect involvement of mitochondria. When compared to normal cells, the cell size, shape and number of mitochondria may vary for cancer cells. Further, cancer cells may also have abnormally elevated level of oxidative enzymes, the elevated level of porphyrin and their biosynthetic pathways in malignant cells and tissues(figure 11) due to excess conversion of PpIX due to the disturbances and alterations in the key enzymes such as, porphobilinogen and ferrochelatase. In malignant cells, the amount of ferrochelatase may be less and hence, there was a slow or minimal conversion of PpIX into heme. Also there can be degradation of amino acid due to alteration in urea cycle indirectly affecting the heme synthesis. In this pathway, the aminoacid L-Argnine were converted into L-Citrulline and a highly reactive nitric oxide was produced as a byproduct due to the action of an enzyme nitric oxide synthase. This reactive species reduces the heme synthesis rate by inhibiting ferrochelatase activity, which results on accumulation of more porphyrin[86] (binactivation of mammalian ferrochelatase in vivo and in vitro: possible involvement of the iron-sulphur cluster of the enzyme. This excess amount of porphyrin induces cell breakage due to oxidative stress leading to release of excess porphyrin in saliva of potentially maliganant disorder and oral cancer patients by pinocytosis and/or

exocytosis from the cells. This also explains the basis of decreased level of porphyrins of patients under treatment as tumour cells are destroyed by radiation.

**Merits of the study:**

Saliva collection is easy, and being a painless procedure repeated samples can be collected from same patient and has better patient acceptibility.

The spectral analysis of saliva can be obtained immediately and diagnosis can be made within a few hours after subjecting the saliva by spectrometry

Since lesions in the oral cavity is always wetted by saliva, the exfoliated cells from dysplastic premalignant cells and exfoliated damaged Oral carcinoma cells shows significant alterations, studies done by Pfaffe, J.C. White et shows the changes in the saliva is about 1000 times more in oral cancer patients when compared to blood or urine as the alteration detected would become feeble when blood distributed throughout the body or may get metabolized at tissue site. So the values from the blood drawn at distant site will be less.

**Limitations of the study** :

The results thus obtained demonstrate the exciting potential for the application of this new technology for the diagnosis of potentially malignant disorders at an early stage and also as an adjuctive tool of prognostic indicator . However, before this technique can be brought to the general public, several major obstacles must be overcome. First, larger clinical trials need to be performed to confirm the preliminary results obtained in the initial clinical trials. Second, the optimal excitation and emission wavelengths needed to differentiate between normal and abnormal tissue at each oral cavity location must be ascertained.

In patients with inflammatory diseases such as periodontitis or pharyngitis there can be significant intruption of the spectrum due to elevated porphyrins and other protiens in saliva due to inflammatory process, break down of RBC with expression of heme and cellular death. Porphyrins can also be produced by iron metabolizing bacteria in periodontium which can lead to false positive results. In patients with large carcinomas there can be area of necrosis and pus discharge due to secondary infection, in such cases the saliva may get contaminated with dead cells and pus thereby bringing false positive results.

In patients with systemic diseases such as diabetes mellitus, and renal diseases there is alterations in viscosity, constituencies and pH of saliva. This leads to alterations in spectrum of saliva and false positive results.in patients with smoking habit there can be alteration in quantity and contituents and pH of saliva leading to false positive results.

In patients under radiotherapy there is practical becomes difficulty in saliva collection as there is marked decrease in salivary flow following radiotherapy after 3$^{rd}$ week. Also when mucositis occurs due to radiation there is contamination of saliva from poor oral hygiene and inflammation of the entire oral mucosa and soft palate results in irregular spectrum with no significant peaks, thus they are of no diagnostic value. Thus the study requires to be done in larger clinical trial with more effective saliva collection method and homogenous sample in radiotherapy patients to accurately assess their prognosis.

**CONCLUSION :**

The current study used laser induced fluorescence spectra of the saliva from oral carcinoma, potentially malignant disorder and radiotherapy patients to assess spectroscopic features associated with carcinoma, premalignant and treatment progression. The rich source of information and diagnostic potential provided by noninvasive spectroscopic techniques allows to understand the changes that take place during the onset and progression of oral carcinoma. The results of the study demonstrated that autofluorescence due to endogenous flurophores such as NADH, FAD, porphyrin changes has excellent diagnostic potential. Because this technique can discriminate between malignant, potentially malignant disorder from healthy mucosa, it could be used as a diagnostic modality to differentiate between malignant and potentially malignant disorder. Also the ability of autofluroscence to detect changes in spectra of malignant and radiotherapy patients shows its potential in detecting prognosis of the treatment. However, the results of our study on autofluorescence characteristics in the diagnosis of malignant and potentially malignant disorders ought to be validated with more studies involving large samples and more homogenous samples before it can be used as a non-invasive confirmatory diagnostic procedure, until such studies are made it can be positively used as an adjunctive diagnostic aid .

## BIBILIOGRAPHY


1. Hari Ram et al Oral Cancer: Risk Factors and Molecular Pathogenesis j. Maxillofac. Oral Surg. (Apr-June 2011) 10(2):132–137.
2. Boring CC, Squires TS, Tong T (1992) Cancer statistics, 1992. Cancer J Clin 42(1):19–38.
3. Jaychandran S, Meenapriya PK, Ganesan S (2016) Raman Spectroscopic Analysis of Blood, Urine, Saliva and Tissue of Oral Potentially Malignant Disorders and Malignancy-A Diagnostic Study Int J Oral Craniofac Sci 2(1): 011-014. DOI: 10.17352/2455-4634.000013.
4. M. Yuvaraj et al. Journal of Photochemistry and Photobiology B: Biology 130 (2014) 153–160.
5. Mortazavi H, Baharvand M, Mehdipour M. Oral Potentially Malignant Disorders: An Overview of More than 20 Entities. Journal of Dental Research, Dental Clinics, Dental Prospects. 2014.
6. S. Warnakulasuriya1, Newell. W. Johnson2, I. van der Waal ,Nomenclature and classification of potentially malignant disorders of the oral mucosa, J Oral Pathol Med (2007) 36: 575–80.
7. Pindborg JJ, Sirsat SM. Oral submucous fibrosis. Oral Surg. Oral Med. Oral Pathol. 1966;22(6):764-79.
8. Schwartz J. Atrophia Idiopathica Mucosae Oris. In: Demonstrated at the 11th Int Dent Congress;1952.
9. Ranganathan K, Devi MU, Joshua E, Kirankumar K, Saraswathi TR. Oral submucous fibrosis: a case-control study in Chennai, South India. J. Oral Pathol. Med. 2004;33(5):274-7.
10. Wahi PN, Kapur VL, Luthra UK, Srivastava MC. Submucous fibrosis of the oral cavity. 1. Clinical features. Bull. World Health Organ. 1966;35(5):789-92.
11. Wahi PN, Kapur VL, Luthra UK, Srivastava MC. Submucous fibrosis of the oral cavity. 1. Clinical features. Bull. World Health Organ. 1966;35(5):789-92.
12. Seedat HA, van Wyk CW. Submucous fibrosis (SF) in ex-betel nut chewers: a report of 14 cases. J. Oral Pathol. 1988;17(5):226-9.
13. Trivedy C, Warnakulasuriya KA, Hazarey VK, Tavassoli M, Sommer P, Johnson NW. The

upregulation of lysyl oxidase in oral submucous fibrosis and squamous cell carcinoma. J. Oral Pathol. Med. 1999;28(6):246-51.

14. Paymaster JC. Cancer of the buccal mucosa.A clinical study of 650 cases in Indian patients. Cancer 1956;9(3):431-435.
15. Mehta FS, Gupta PC, Daftary DK, Pindborg JJ, Choksi SK. An epidemiologic study of oral cancer and precancerous conditions among 101,761 villagers in Maharashtra, India. Int. J. Cancer 1972;10(1):134-41.
16. Murti PR, Bhonsle RB, Pindborg JJ, Daftary DK, Gupta PC, Mehta FS. Malignant transformation rate in oral submucous fibrosis over a 17-year period. Community Dent. Oral Epidemiol. 1985;13(6):340-1.
17. Kramer IR, Lucas RB, Pindborg JJ, Sobin LH. Definition of leukoplakia and related lesions: An aid to studies on oral precancer. Oral Surg Oral Med Oral Pathol 1978;46:518-39.
18. Axéll T, Pindborg JJ, Smith CJ, van der Waal I. Oral white lesions with special reference to precancerous and tobacco - Related lesions: Conclusions of an International Symposium held in Uppsala, Sweden, May 18-21 1994. International Collaborative Group on Oral White Lesions.
19. S. Warnakulasuriya 1, Newell. W. Johnson2, I. van der Waal ,Nomenclature and classification of potentially malignant disorders of the oral mucosa, J Oral Pathol Med (2007) 36: 575–80)
20. Feller L, Lemmer J. Oral leukoplakia as it relates to HPV infection: A review. Int J Dent 2012;2012:540561.)
21. S. Warnakulasuriya 1, Newell. W. Johnson2, I. van der Waal ,Nomenclature and classification of potentially malignant disorders of the oral mucosa, J Oral Pathol Med (2007) 36: 575–80
22. Gupta PC, Mehta FS, Pindborg JJ, Bhonsle RB, Murti PR, Daftary DK, et al. Primary prevention trial of oral cancer in India: A 10-year follow-up study. J Oral Pathol Med 1992;21:433-9.].
23. Feller L, Lemmer J. Oral leukoplakia as it relates to HPV infection: A review. Int J Dent

2012;2012:540561.

24. Downer MC, Petti S. Leukoplakia prevalence estimate lower than expected. Evid Based Dent 2005;6:12]
25. Martorell-Calatayud A, Botella-Estrada R, Bagán-Sebastián JV, Sanmartín-Jiménez O, Guillén-Baronaa C. Oral leukoplakia: Clinical, histopathologic, and molecular features and therapeutic approach. Actas Dermosifiliogr 2009;100:669-84.
26. Brouns ER, Baart JA, Bloemena E, Karagozoglu H, van der Waal I. The relevance of uniform reporting in oral leukoplakia: Definition, certainty factor and staging based on experience with 275 patients. Med Oral Patol Oral Cir Bucal 2013;18:e19-26.
27. Bánóczy J. Follow-up studies in oral leukoplakia. J Maxillofac Surg 1977;5:69-75.
28. Kayalvizhi EB, Lakshman VL, Sitra G, Yoga S, Kanmani R, Manimegalai. Oral leukoplakia: A review and its update. J Med Radiol Pathol Surg 2016;2:18-22.
29. Axéll T, Pindborg JJ, Smith CJ, van der Waal I. Oral white lesions with special reference to precancerous and tobacco - Related lesions: Conclusions of an International Symposium held in Uppsala, Sweden, May 18-21 1994. International Collaborative Group on Oral White Lesions. J Oral Pathol Med 1996;25:49-54..
30. Brouns ER, Baart JA, Bloemena E, Karagozoglu H, van der Waal I. The relevance of uniform reporting in oral leukoplakia: Definition, certainty factor and staging based on experience with 275 patients. Med Oral Patol Oral Cir Bucal 2013;18:e19-26.
31. Roed-Petersen B, Gupta PC, Pindborg JJ, Singh B. Association between oral leukoplakia and sex, age, and tobacco habits. Bull World Health Organ 1972;47:13-9
32. Bánóczy J. Follow-up studies in oral leukoplakia. J Maxillofac Surg 1977;5:69-75.
33. Schepman KP, Bezemer PD, van der Meij EH, Smeele LE, van der Waal I. Tobacco usage in relation to the anatomical site of oral leukoplakia. Oral Dis 2001;7:25-7
34. Brzak BL, Mravak-Stipetic M, Canjuga I, Baricevic M, Balicevic D, Sikora M, et al. The

frequency and malignant transformation rate of oral lichen planus and leukoplakia – A retrospective study. Coll Antropol 2012;36:773-7.

35. George A, BSS, SS, Varghese SS, Thomas J, Gopakumar D, Mani V. Potentially Malignant disorders of oral cavity. OMPJ 2011;2:95-100.
36. Neville BW, Damm DD, Allen CR, Bouquot JE. Oral and maxillofacial pathology. 2nd ed. Philadelphia: WB Saunders; 2002. P. 316-376, 644- 697.
37. Fernández A, Córdova P, Badenier O & Esguep A. Epidemiological characterization of oral cancer. Literature review. J Oral Res 2015; 4(2): 137-145.
38. CD Llewlyn, et al, risk factors for oral squamous cell carcinoma in young people-a comprehensive literature review, oral oncology 31(2001) 401-408.
39. McCoy GD (1978) A biochemical approach to the aetiology of alcohol related cancers of the head and neck. Laryngoscope 88:59–62.
40. Ram, Hari et al. "Oral Cancer: Risk Factors and Molecular Pathogenesis." Journal of Maxillofacial & Oral Surgery 10.2 (2011): 132–137. PMC. Web. 12 Dec. 2016.
41. Kreimer AR, Clifford GM, Boyle P, Franceschi S (2005) Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol Biomarkers Prev 14(2):467–475.
42. Parkin DM, Bray F, Ferlay J, Pisani P (2005) Global cancer statistics-2002. CA Cancer J Clin 55(2):74–108, J. Maxillofac. Oral Surg. (Apr-June 2011) 10(2):132–137 .
43. Cawson RA, Binnie WH (1980) Candida, leukoplakia and carcinoma: a possible relationship. In: Mackenzie IC, Dabelsteen E, Squier CA (eds) Oral premalignancy, 1st edn. University of Iowa Press, Iowa city, pp 59–66
44. Wahi PN, Kehar U, Lahiri B (1965) Factors influencing oral and oropharyngeal cancer in India. Br J Cancer 19(4):642–660.

45. Notani PN, Sanghvi LD (1976) Role of diet in the cancer of the oral cavity. Indian J Cancer 13(2):156–160]

46. Epstein JB, Gorsky M, Lonky S, Silverman S Jr, Epstein JD, Bride M. The efficacy of oral lumenoscopy (ViziLite) in visualizing oral mucosal lesions. Spec Care Dentist 2006;26:171-174.

47. Rosin MP, Poh CF, Guillard M, Williams PM, Zhang L, Macaulay C. Visualization and other emerging technologies as change makers for oral cancer prevention. Ann N Y AcadSci 2007;1098:167-183

48. Jayachandran, et al. Spectroscopic analysis of oral premalignancy and malignancy, Journal of Indian Academy of Oral Medicine and Radiology, April-June 2009, Volume 21, Issue 2 PG 55-63.

49. Jaychandran S, Meenapriya PK, Ganesan S (2016) Raman Spectroscopic Analysis of Blood, Urine, Saliva and Tissue of Oral Potentially Malignant Disorders and Malignancy-A Diagnostic Study. Int J Oral Craniofac Sci 2(1): 011-014.

50. Nauta JM, van Leengoed HL, Star WM, Roodenburg JL, Witjes MJ, Vermey A. Photodynamic therapy of oral cancer. A review of basic mechanisms and clinical applications. Eur J Oral Sci 1996;104:69–81.

51. Profio AE, Doiron DR. A feasibility study of the use of fluorescence bronchoscopy for localization of small lung tumors. Phys Med Biol 1977;22:949–57.

52. Wang CY, Tsai T, Chen HC, Chang SC, Chen CT, Chiang CP. Autofluorescence spectroscopy for in vivo diagnosis of DMBA-induced hamster buccal pouch pre-cancers and cancers. J Oral Pathol Med2003;32:18-24.

53. Dhingra JK, Zhang X, McMillan K, Kabani S, Manoharan R, Itzkan I, Feld MS, Shapshay SM. Diagnosis of head and neck precancerous lesions in an animal model using fluorescence spectroscopy. Laryngoscope 1998;108 (4 Pt 1): 471-475.

54. Zheng W, Soo KC, Sivanandan R, Olivo M. Detection of neoplasms in the oral cavity by digitized endoscopic imaging of 5-aminolevulinic acid-induced protoporphyrin IX fluorescence. Int J Oncol 2002;21:763-768.
55. Leunig A, Betz CS, Baumgartner R, Grevers G, Issing WJ. Initial experience in the treatment of oral leukoplakia with high-dose vitamin A and follow-up 5-aminolevulinic acid induced protoporphyrin IX fluorescence. Eur Arch Otorhinolaryngol 2000;257:327-331.
56. Auluck A, Rosin MP, Zhang L, Sumanth KN. Osmf a clinically beningn but potentially malignant diseases.Report of 3 cases & review literature. J.canadian association 2008:74(8):735-40
57. Kumar Kiran. Sarasvvathi TR, Rangnathan K. Devi Lima M, Elizabeth Joshua. Oral submucous fibrosis: A clinico- histopathological study in Chennai. Indian Journal of Denial Research 2007:18(3): 106-11)
58. Rajalalitha P, Vali S. Molecular pathogenesis of oral submucous fibrosis--a collagen metabolic disorder. J. Oral Pathol. Med. 2005;34(6):321-8.
59. N Dyavanagoudar S. Oral Submucous Fibrosis: Review on Etiopathogenesis. J. Cancer Sci. Ther. 2009;01(02):072-077.
60. Wanninayake Mudiyanselage Tilakaratne RPE. Oral Submucous Fibrosis: Review on Mechanisms of Pathogenesis and Malignant Transformation. J. Carcinog. Mutagen. 2013.
61. Ma RH, Tsai CC, Shieh TY. Increased lysyl oxidase activity in fibroblasts cultured from oral submucous fibrosis associated with betel nut chewing in Taiwan. J. Oral Pathol. Med. 1995;24(9):407-12.
62. Yadav J. Role of Copper in Oral Submucous Fibrosis: A Cytological Correlation. Indian J. Dent. Sci. 2011;3(5):3-6.

63. Tsai C-H, Chou M-Y, Chang Y-C. The up-regulation of cyclooxygenase-2 expression in human buccal mucosal fibroblasts by arecoline: a possible role in the pathogenesis of oral submucous fibrosis. J. Oral Pathol. Med. 2003;32(3):146-53.
64. Haque MF, Meghji S, Khitab U, Harris M. Oral submucous fibrosis patients have altered levels of cytokine production. J. Oral Pathol. Med. 2000;29(3):123-8.
65. Rajendran R, Paul S, Mathews PP, Raghul J, Mohanty M. Characterisation and quantification of mucosal vasculature in oral submucous fibrosis. Indian J. Dent. Res. 16(3):83-91.
66. Rajendran R, Sunil, Twinkle SP, Anikumar T V, Annie J. Cell death does not herald epithelial involution ("atrophy") in oral sub mucous fibrosis: a TEM study. Indian J. Dent. Res. 15(1):13-9.
67. Rajendran R, Varkey S. Inducible nitric oxide synthase expression is upregulated in oral submucous fibrosis. Indian J. Dent. Res. 18(3):94-100.
68. Thangjam GS, Agarwal P, Khan I, et al. Transglutaminase-2 regulation by arecoline in gingival fibroblasts. J. Dent. Res. 2009;88(2):170-5.
69. Tsai C-H, Yang S-F, Lee S-S, Chang Y-C. Augmented heme oxygenase-1 expression in areca quid chewing-associated oral submucous fibrosis. Oral Dis. 2009;15(4):281-6.
70. Gupta P, R Naik S, Nc S, Durgvanshi A, Agarwal N. Salivary IgA Levels in Patients with Oral Submucous Fibrosis: A Study. Kailasam S, ed. J. Indian Acad. Oral Med. Radiol. 2011;23(4):536-538.
71. Williams HK (2000) Molecular pathogenesis of oral squamous carcinoma. Mol Pathol 53(4):165–172
72. Brennan P, Lewis S, Hashibe M, Bell DA, Botteffa D, Bouchardy C et al (2004) Pooled analysis of alcohol dehydrogenase genotypes and head and neck cancer—review. Am J Epidemiol 159(1):1–16.

73. Shao X, Tandon R, Samara G, Kanki H, Yano H, Close LG et al (1998) Mutational analysis of PTEN in head and neck squamous cell carcinoma. Int J Cancer 77(5):684–688.

74. Mao L, Lee JS, Fan YH, Ro JY, Batsakis JG, Lippman S et al (1996) Frequent microsatellite alterations at chromosome 9p21 and 3p14 in oral premalignant lesions and their value in cancer risk assessment. Nat Med 2(6):682–685.

75. Tseng JE, Kemp BL, Khuri FR, Kurie J, Lee JS, Zhou X et al (1999) Loss of Fhit is frequent in stage I non small cell lung cancer and in the lung of chronic smokers. Cancer Res 59(19):4798–4803.

76. Spafford MF, Koch WM, Reed AL, Califano JA, Xu LH, Eisenberger CF et al (2001) Detection of head and neck squamous cell carcinoma among exfoliated oral mucosal cells by microsatellite analysis. Clin Cancer Res 7(3):607–612.

77. Califano J, Van der Riet P, Westra W, Nawroz H, Clayman G, Piantadosi S et al (1996) Genetic progression model for head and neck cancer: implications for field cancerization. Cancer Res 56(11):2488–2492.

78. Brennan JA, Boele JO, Koch WM, Goodman SN, Hruban RH, Eby YJ et al (1995) Association between cigarette smoking and mutation of the p53 gene in head and neck squamous cell carcinoma. N Engl J Med 332(11):712–717.

79. Devita VT Jr, Lawrence TS, Rosenberg SA (eds) (2008) Cancer principals and practice of oncology, vol 1, 8th edn. Lippincott-Williams and Wilkins, Philadelphia, pp 799–808.

80. Vedeswari C P, Jayachandran S, Ganesan S. In vivo autofluorescence characteristics of pre- and post-treated oral submucous fibrosis: A pilot study. Indian J Dent Res 2009;20:261-7.

81. R. Monteforte, A. Santillo, M. Di Giovanni, A.D. Aniello, A. Di Maro, G. Chieffi Baccari, D-Aspartate affects secretory activity in rat Harderian gland:molecular mechanism and functional significance, Amino Acids 37 (4) (2009) 653–664.

82. Svistun E, Alizadeh-Naderi R, El-Naggar A, Jacobs R, Gillenwater A, Richards Kortum. Vision enhancement system for detection of oral cavity neoplasia based on autofluorescence. Head Neck 2004;26:205-215.

83. Balevi B. Assessing the usefulness of three adjunctive diagnostic devices for oral cancer screening: a probabilistic approach. Community Dent Oral Epidemiol 2011;39:171–176.

84. Lakowicz J R. Principles of Fluorescence Spectroscopy. New York: Kluwer Academic/Plenum,1999.

85. Tatjana Dramićanin and Miroslav Dramićanin Using Fluorescence Spectroscopy to Diagnose Breast Cancer. Applications of Molecular Spectroscopy to Current Research in the Chemical and Biological Sciences, chapter 2, pg 261- 282

86. Silverman S, Jr. Radiation effects. In Silverman S, Jr ed. Oral cancer. New York: American Cancer Society, 1990:81-90.

87. Sreebny L. Xerostomia: diagnosis, management and clinical complications. In Edgar WM, O'Mullane DM, eds. Saliva and Oral Health, 2nd edn. London: BDJ, 1996:43-66.

TAMIL NADU GOVRNMENT DENTAL COLLEGE & HOSPITAL, CHENNAI – 3.

TELEPHONE : 044-253403343
FAX: 044- 25300681
date : 26/09/2015

Ref No: R.C No.0430/DE/2015 dated 27.01.2015, O/O Principal, TNGDC
Sub: IEC review of the research proposals,

Title of the work: Auto fluorescence spectroscopic analysis of salivary metabolites in patients with potentially malignant disorders, oral cancer patients and in patients under radiotherapy

Principal Investigator: Dr.S. Senthil Kumar
II year M.D.S., student

Department : Department of Oral Medicine and Radiology
Tamil Nadu Govt. Dental College & Hospital , Chennai-3

Thank you for submitting your research proposal , which was considered at the Institutional Ethics Committee meeting held on 02-07-2015, at TN Govt. Dental College and the documents related to the study referred above were discussed and the modifications done as suggested and reported to us through your letter dated 25-09-2015 have been reviewed.

The decision of the members of the committee , the secretary and the Chairperson IEC of TN Govt. Dental College is here under:

| | |
|---|---|
| Approved | Approved and advised to proceed with the study |
| Approved with suggestions | ---------- |
| Revision | ---------- |
| Rejected | ---------- |

The principal investigators and their team are advised to adhere the guide lines given below:

1. You should get detailed informed consent from the patients / participants and maintain confidentiality.
2. You should carry out the work without affecting regular work and without extra expenditure to the Institution or the Government.
3. You should inform the IEC, in case of any change of study procedure, site, and investigating guide.
4. You should not deviate from the area of work for which you have applied for ethical clearance.
5. You should inform the IEC immediately in case of any adverse events or serious adverse reactions. You should abide to the rules and regulations of the institution(s) .
6. You should complete the work within specific period and if any extension of time is required, you should apply for permission again to do the work.
7. You should submit the summary of the work to the ethical committee every 3 months and on completion of the work.
8. You should not claim any kind of funds from the institution for doing the work or on completion/ o for any kind of compensations.
9. The members of the IEC have the right to monitor the work without prior intimation.
10. Your work should be carried out under the direct supervision of the guide/ Professor.

MEMBER SECRETARY,
INSTITUTIONAL ETHICS COMMITTEE
Tamil Nadu Govt. Dental College & Hospital
Chennai

CHAIRPERSON
INSTITUTIONAL ETHICS COMMITTEE
Tamil Nadu Govt. Dental College & Hospital
Chennai

**PARTICIPANT INFORMATION SHEET**

STUDY TITLE: "**Autofluorescence spectroscopic analysis of salivary metabolites in patients with potentially malignant disorders ,oral cancer patients and in patients under radiotherapy"** .

Name Of The Research Instituition:

1. Tamilnadu Government Dental College & Hospital, Chennai-03.
2. Anna university ,Department of Medical Physics, Guindy- 600032
3. Rajiv Gandhi General hospital ,Radiation oncology,Chennai-01

1. **Purpose of the study**:

To evaluate spectroscopic intensity of saliva in potentially malignant disorders,oral cancer patients and patient under radiotherapy, and to compare autofluorescene characteristics in premalignant, malignant and patients under radiotherapy.

2. **Procedures** :

I. Patient selection.

II. Obtaining Thorough history and informed consent.

III. Complete Clinical Examination (intra and extra oral examination) by using diagnostic instrument set.

IV. A small fragment of the tissue from the site of the lesion will be taken for analysis. The tissue will be harvested in sterile condition, and post surgical care and follow up will be done .

V. Histopathological analysis is carried out for Leukoplakia and oral cancer patients only, patients with Oral submucous Fibrosis are clinically diagnosed.

VI. Patients saliva is collected in a sterile container and subjected to Autofluorescence spectroscopic analysis.

VII. From the results obtained accurate diagnosis, prognosis of the disease can be evaluated.

**3 Risk of participation**:

Patients are selected only by proper inclusion and exclusion criteria so ,and as the procedure is minimally invasive the risk of participation is minimal.

**4. Benefits**:

Patients will be benefited by early diagnosis of the disease by a minimally-invasive procedure and also assessment of prognosis of the oral cancer patients under radiotherapy can be evaluated.

**5. Confidentiality**:

The identity of the patients participating in the research will be kept confidential throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

**6. Participant's rights:**

a).Taking part in the study is voluntary. You are free to decide whether to participate in the study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.

b).The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

7. Compensation**:** Nil

8. Contacts for queries related to the study:

Primary Investigator name : Dr.S.Senthil Kumar

Contact details :Department of Oral Medicine ad Radiology,

Tamil Nadu government Dental college and Hospital,

Frazer Bridge Road,

Chennai-600003

Phone number- 9500425394

(For queries related to the rights as a study participant, please write to: The Chairperson, IEC-Tamil nadu government dental college and Hospital ,Chennai-600003)

## ஆராய்ச்சி பற்றிய தகவல் படிவம்

வாய் முன் புற்று நோய் அறிகுறி உடையவர், வாய் புற்று நோயாளி மற்றும் கதிர்வீச்சு சிகிச்சை அளிக்கப்பட்ட நோயாளியின் உமிழ் நீரை தன்மிளிர்வு பரிசோதனை மூலம் நடத்தப்படும் ஆய்வு என்ற ஆய்வை தமிழ்நாடு அரசு பல் மருத்துவக் கல்லூரி மருத்துவமனைக்கு வரும் நோயாளிகளிடம் நடத்த உள்ளோம். அதற்காக மேற்கண்ட நோயாளிகளை தேர்வு செய்கிறோம்.

வாய் முன்புற்றுநோய் மற்றும் புற்றுநோய் உடைய நோயாளியின் தசை பரிசோதனை செய்து நோய் கண்டறியப்படும். நோயாளியின் 5 முதல் 10 மில்லி உமிழ்நீர் காலைவேளையில் சேகரிக்கப்படும் மற்றும் கதிர்வீச்சு சிகிச்சை பெறும் நோயாளியின் 5 முதல் 10 மில்லி உமிழ்நீர் காலைவேளையில் சேகரிக்கப்பட்டு தன்மிளிர்வு மூலம் பரிசோதனை செய்யப்படும்.

நோயாளிகள் பற்றிய குறிப்புகள் ஆராய்ச்சி முடியும்வரை ரகசியமாக பாதுகாக்கப்படும். இந்த ஆராய்ச்சியை வெளியிடும்போது நோயாளிகளின் தனிப்பட்ட விவரங்கள் எதுவும் பாதிக்கப்படமாட்டாது.

இந்த ஆராய்ச்சியில் பங்கு பெறுவது நோயாளியின் தனிப்பட்ட விருப்பம், மேலும் நோயாளிகள் இந்த ஆராய்ச்சியிலிருந்து எப்போது வேண்டுமென்றாலும் விலகிக்கொள்ளலாம். நோயாளியின் இந்த முடிவினால் அவருக்கோ அல்லது ஆராய்ச்சியாளருக்கோ எந்தவித பாதிப்பும் கிடையாது.

இந்த ஆராய்ச்சியின் முடிவுகள் நோயாளிகளுக்கு ஆராய்ச்சியின் இடையிலோ அல்லது முடிவிலோ தெரிவிக்கப்படும். இதில் ஏதேனும் பின் விளைவுகள் ஏற்பட்டால் அதை சரி செய்ய அளிக்க தகுந்த உதவிகள் செய்யப்படும்.

**ஆய்வாளரின் கையொப்பம்** **பங்கேற்பாளர் கையொப்பம்**

தேதி :
இடம் :

**Annexure : AF 06/004/01.0**

**INFORMED CONSENT FORM**

**STUDY TITLE:**

**Autofluorescence spectroscopic analysis of salivary metabolites in patients with potentially malignant disorders ,oral cancer patients and in patients under radiotherapy**

Name: Age / Sex: O.P.No:

Address: Serial No:

Tel. no:

I,_____age____years

- Exercising my free power of choice, hereby give my consent to be included as a participant in the study "**Autofluorescence spectroscopic analysis of salivary metabolites in patients with potentially malignant disorders ,oral cancer patients and in patients under radiotherapy".**

  I agree to the following:

- I have been informed to my satisfaction about the purpose of the study and study procedures including investigations to monitor and safeguard my body function.

- I agree to cooperate fully and to inform my doctor immediately if I suffer any unusual symptom.

- I have informed the doctor about all medications I have taken in the recent past and those I am currently taking and other systemic illness that I have.

- I agree to report to the doctor for a regular follow-up as and when required for the research.

- I hereby give permission to use my medical records for research purpose. I am told that the investigating doctor and institution will keep my identity confidential.

Name of the patient Signature / Thumb impression

Name of the investigator Signature

Date:

Place :

## சுய ஒப்புதல் படிவம்

## ஆய்வு செய்யப்படும் தலைப்பு

**வாய் முன் புற்று நோய் அறிகுறி உடையவர், வாய் புற்று நோயாளி மற்றும் கதிர்வீச்சு சிகிச்சை அளிக்கப்பட்ட நோயாளியின் உமிழ் நீரை தன்மிளிர்வு பரிசோதனை மூலம் நடத்தப்படும் ஆய்வு**

ஆராய்ச்சி நிலையம் : அரசு பல் மருத்துவக் கல்லூரி

சென்னை-600 003.

பங்கு பெறுபவரின் பெயர் :

பங்கு பெறுபவரின் பிறந் தேதி :

தேதி மாதம் வருடம்

இப்படிப்பு சம்பந்தமாக நான் மேலே கூறப்பட்ட தகவல் படிவத்தை முழுமையாக படித்துப் பார்த்தேன் என்று உறுதி கூறுகிறேன்.

நான் இது தொடர்பான அனைத்து கேள்விகளுக்கும் நிறைவான பதில்கள் பெறப்பட்டன.

இந்த ஆய்வின் எனது பங்கு தன்னிச்சையானது என்றும் எந்த நேரத்திலும் இந்த ஆய்வில் இருந்து சட்ட உரிமைகள் பாதிக்கப்பட்டால் விலகிக் கொள்ள சம்மதிக்கிறேன்.

மருத்துவ ஆய்வு அதிகாரிகள், எனது சிகிச்சை தொடர்பான பதிவேடுகளை பார்வையிடவும் எந்த நேரத்திலும், ஆய்வில் இருந்து நான் விலகினாலும் பார்வையிட சம்மதிக்கிறேன். எனது அடையாள குறிப்புகள் மூன்றாவது நபருக்கு தெரிவிக்கப்படமாட்டாது என்று புரிந்து கொண்டேன்.இந்த ஆய்வு அறிக்கைகளை பயன்படுத்தவும், வெளியிடவும், நான் சம்மதிக்கிறேன். ஆய்வாளர் எனது மருத்துவக் குறிப்புகளை வெளியிட தடையாக இருக்கமாட்டேன் என உண்மையாக சம்மதிக்கிறேன்.
வாய் முன்புற்றுநோயாளியாகிய நான் / வாய் புற்றுநோயாளியாகிய நான் / கதிர்வீச்சு சிகிச்சை பெறும் நோயாளியாகிய நான் /இந்த ஆய்வுக்கு முழுமையாக சம்மதிக்கிறேன்.

பங்கேற்பவரின் கையொப்பம் ..... இடம் ..... தேதி .....

கட்டைவிரல் நேரகை
பங்கேற்பவரின் பெயர் மற்றும் விலாசம் .....

ஆய்வாளரின் கையொப்பம் ..... இடம் ..... தேதி .....

ஆய்வாளரின் பெயர் .....

**DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY**

**TAMIL NADU GOVT. DENTAL COLLEGE & HOSPITAL, CHENNAI -3**

**STUDY PROFOMA**

Date: Serial no:

Name: O.P No:

Age/Sex:

Address:

Phone no:

Occupation: Income:

:

Presenting complaint with duration:

Past medical history:

Past Surgical history:

Past dental history:

Personal history:

A) Diet:

B) Teeth cleaning habits:

- Cleaning aids used:
- Frequency :

C) Smoking habit:

- Material used:
- Frequency :
- Duration of the habit:

D) Chewing habit:

- Material used:
- Frequency :
- Duration of the habit:

E) Other habits (alcohol, snuff):

Marital status:

Family history:

**CLINICAL EXAMINATION**

Extraoral Examination:

Intraoral examination:

- Mouth opening (Inter incisal distance):

- Labial and buccal mucosa:

- Hard and soft palate, Uvula:

- Tongue:

- Floor of the mouth:

- Retromolar trigone:

Clinical diagnosis:

**Investigations:**

1) Laboratory investigations:

A) Blood:

Total WBC count:

Erythrocyte sedimentation rate:

Differential count:

Haemoglobin %:

Clotting time:

Bleeding Time:

Peripheral smear:

B) Urine:

Glucose:

Albumin:

Deposits:

2) Radiological Examination:

3) Histopathology:

4) Autofluorescence spectroscopic analysis:

.

| salivary metabolites: | Emission spectrum At 405 nm | Emission spectrum At 620 nm |
|---|---|---|
| Group I- potentially malignant patients saliva | | |
| Group II- oral cancer patient's saliva | | |
| Group III- patient under radiotherapy | | |
| Group IV- Control group | | |

Final diagnosis:

Treatment plan:

# TRIPARTITE AGREEMENT

This agreement hereinafter the "Agreement" is entered into on this day……. between the Tamil Nadu Government Dental College and Hospital represented by its **Principal** having address at Tamil Nadu Government Dental College and Hospital, Chennai- 600 003, (hereinafter referred to as, 'the college') And

**Dr. S. JAYACHANDRAN, M.D.S., Ph.D.,** aged 53 years working as **Professor and HOD** in Department of Oral medicine and Radiology at the college, having residence address at A.M -16, TNHB quarters,TOD Hunter nagar, saidapet ,Chennai – 15.(herein after referred to as the 'Principal Investigator') And

**Dr.S. SENTHIL KUMAR ,** aged 33 years currently studying as final year **Postgraduate student** in the Department of Oral Medicine and Radiology, Tamil Nadu Government Dental College and Hospital, Chennai -3 ( hereafter referred to as the 'PG and co- investigator') residing at no.T-158, Cholan Street, Paari Nagar, jafferkhanpet, Chennai- 600083

Whereas the 'PG student as part of his curriculum undertakes to research on" for which purpose the Principal investigator shall act as Principal investigator and the College shall provide the requisite infrastructure based on availability and also provide facility to the PG student as to the extent possible as a Co-investigator

Whereas the parties, by this agreement have mutually agreed to the various issues including in particular the copyright and confidentiality issues that arise in this regard

Now this agreement witnessed as follows :

1. The parties agree that all the Research material and ownership therein shall become the vested right of the college, including in particular all the copyright in the literature including the study, research and all other related papers.

2. To the extent that the college has legal right to do go, shall grant to licence or assign the copyright so vested with it for medical and/or commercial usage of interested persons/entities subject to a reasonable terms/conditions including royalty as deemed by the college.

3. The Royalty so received by the college shall be shared equally by all the three parties.

4. The PG/Research student and PG/Principal Investigator shall under no circumstances deal with the copyright, Confidential information and know-how-generated during the course of research/study in any manner whatsoever, while shall sole west with the college.

5. The PG student and Principal Investigator undertake not to divulge (or) cause to be divulged any of the confidential information or, know-how to anyone in any manner whatsoever and for any purpose without the express written consent of the college.

6. All expenses pertaining to the research shall be decided upon by the principal investigator/Co-investigator or borne sole by the PG student.(co-investigator)

7. The college shall provide all infrastructure and access facilities within and in other institutes to the extent possible. This includes patient interactions, introductory letters, recommendation letters and such other acts required in this regard.

8. The Principal Investigator shall suitably guide the Student Research right from selection of the Research Topic and Area till its completion. However the selection and conduct of research, topic and area research by the Student Researcher under guidance from the Principal Investigator shall be subject to the prior approval, recommendations and comments of the Ethical Committee of the College constituted for this purpose.

9. It is agreed that as regards other aspects not covered under this agreement, but which pertain to the research undertaken by the PG student, under guidance from the Principal Investigator, the decision of the College shall be binding and final.

10. If any dispute arises as to the matters related or connected to this agreement herein, it shall be referred to arbitration in accordance with the provisions of the Arbitration and Conciliation Act, 1996.

In witness where of the parties herein above mentioned have on this the day month and year herein above mentioned set their hands to this agreement in the presence of the following two witnesses.

College represented by its **Principal** **PG Student**

Witnesses **Student Guide**

1.

2.

**MASTER CHART 1- POTENTIALLY MALIGNANT DIORDERS**

| S.NO | NAME | AGE | SEX | Frequency of smoking | Duration of smoking | Frequency of betel quid habit | Duration of betel quid habit | Site | Clinical diagnosis | Diagnosis |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Ramakrishnan | 43 | M | - | - | 2-3pkt | 4yrs | Right and left buccal mucosa | leukoplakia | leukoplakia |
| 2. | Maari | 48 | M | | | 5-6 pkt | 15 yrs | Right and left buccal mucosa | OSMF | OSMF |
| 3. | Jaganathan | 45 | M | - | | | | | Verrucous leukoplakia | Verrucous leukoplakia |
| 4. | Thaslim | 45 | M | 6-7 /day | 32yrs | 1pkt | 1.5yrs | Right Buccal mucosa | leukoplakia | leukoplakia |
| 5. | Balasundaram | 55 | M | 20/day | 20yrs | occasionally | 1yrs | Left buccal mucosa, lower lip | leukoplakia | leukoplakia |
| 6. | Annamalai | 51 | M | 2rolls of bidi | 25yrs | -- | - | Left lateral border tongue | leukoplakia | leukoplakia |
| 7. | Malliga | 42 | F | - | - | Occasionally With slaked lime | 15yrs | Left buccal mucosa, palate, alveolar mucosa | Proliferative verrucous leukoplakia | Leukoplakia with mild Epithelial dysplasia |
| 8. | Hrudra Nayak | 49 | M | - | - | 10pkt/day | 12yrs | Left buccal mucosa | Verrucous leukoplakia | leukoplakia |
| 9. | Gajendran | 70 | M | - | | 4pkt/day | 9yrs | Left buccal mucosa | leukoplakia | leukoplakia |
| 10. | Vijayakumar | 49 | M | - | - | 6pkt/day | 4yrs | Left lateral border tongue | leukoplakia | leukoplakia |
| 11. | Kailammal | 60 | F | - | - | 2-3pkt | 14yrs | Right Buccal mucosa | Verrucous leukoplakia | leukoplakia |
| 12. | Rajeshwari | 45 | F | | | 5-6 pkt | 5 yrs | Dorsum and left lateral | leukoplakia | leukoplakia |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | border tongue | | |
| 13. | Bakthavatchalam | 62 | M | 2-3/day | 10yrs | Tobacco powered with betel | 4yrs | Comissures right side | leukoplakia | leukoplakia |
| 14. | MARI | 42 | F | - | - | 10pkt/day | 10yrs | Right Buccal mucosa | OSMF | OSMF |
| 15. | Thambura | 50 | F | - | - | 5pkt/day | 4 yrs | Buccal mucosa | OSMF | OSMF |
| 16. | Antony samy | 44 | M | - - | - | 2pkt/day | 10yrs | B/L Buccal mucosa | Verrucous leukoplakia | leukoplakia |
| 17. | Syed Thomas | 29 | M | 10/day | 5yrs | 6pkt/day | 15yrs | Left buccal mucosa, | OSMF and leukoplakia | OSMF and leukoplakia |
| 18. | Mathivanan | 51 | M | 5/day | 12yrs | 10pkt/day | 6yrs | B/L buccal mucosa, palate | OSMF | OSMF |
| 19. | Philip | 40 | M | - | - | 15/day | 10yrs | B/L Buccal mucosa, tongue | OSMF | OSMF |
| 20. | Venkatesan | 44 | M | 2/day | 2yrs | 6pkt/day | 8yrs | B/L Buccal mucosa | OSMF | OSMF |
| 21. | Manimegalai | 60 | f | - | - | 12pkt/day | 3yrs | B/L Buccal mucosa, palate | OSMF | OSMF |
| 22. | Saravanan | 31 | M | - | - | Snuff dipping | 6yrs | B/L Buccal mucosa, tongue | OSMF | OSMF |
| 23. | Mahesh | 20 | M | - | - | 3-4pkt/day | 3yrs | B/L Buccal mucosa | OSMF | OSMF |
| 24. | Susila | 45 | F | - | | 6-7pkt/day | 4yrs | B/L Buccal mucosa, | OSMF | OSMF |
| 25. | kailash | 40 | M | - | - | Tobacco powered with betel | 4yrs | B/L Buccal mucosa, soft palate | OSMF | OSMF |

MASTER CHART FOR GROUP II

| S.NO | Name | AGE | SEX | Frequency of smoking | Duration of smoking | Frequency of betel quid and or smokeless tobacco habit | duration of betel quid and /smokeless tobacco habit | Site | Clinical diagnosis | Diagnosis | TNM staging |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Dayalan | 50 | M | - | - | 2-3pkt | 4yrs | Right buccal mucosa | Malignant growth | Well differentiated SCC | $T_2N2aM0$ |
| 2. | Umaya | 45 | F | | | 5-6 pkt | 15 yrs | Left buccal mucosa and mandible, skin of cheek | Malignant growth | Well differentiated SCC | T4N2aM0 |
| 3. | Lakshmi | 48 | F | - | | | | Gingivobuccal complex left side | Malignant ulcer | Well differentiated SCC | T3N0MO |
| 4. | Boopathi | 60 | m | 6-7 /day | 32yrs | 1pkt | 16yrs | Left buccal mucosa | Malignant growth | Well differentiated SCC | T2NoMo |
| 5. | Gunaselan | 60 | M | 20/day | 20yrs | occasionally | 10yrs | Palate | Malignant ulcer | Moderately differentiated SCC | T2N0M0 |
| 6. | Mani | 65 | M | 2rolls of bidi | 25yrs | -- | - | Right lateral border tongue | Malignant ulcer | Moderately differentiated SCC | T4N2bM0 |
| 7. | Pitchaiammal | 50 | F | Bidi 3/day | 35yrs | Occasionally With slaked lime | 15yrs | Right lateral border tongue | Malignant ulcer | Well differentiated SCC | T2N0M0 |
| 8. | Srinivasan | 56 | M | - | - | 10pkt/day | 12yrs | Right lateral border tongue | Malignant ulcer | Well differentiated SCC | T3N0M0 |
| 9. | Chellamal | 57 | F | - | | 4pkt/day | 9yrs | Right buccal mucosa | Malignant ulcer | Well differentiated SCC | T3N0M0 |
| 10. | Kamala | 50 | F | - | - | 6pkt/day | | Upper and lower lip | Malignant growth | Well differentiated SCC | T3N2aM0 |
| 11. | Gopal | 60 | M | 2/day | 10yrs | 10pkt/day | 5yrs | lower lip and tongue | Malignant growth and leukoplakia tongue | Well differentiated SCC | T3N2aM0 |
| 12. | Antony | 47 | M | - | - | 4-6 pkt/day | 13yrs | Right retromolar trigone and buccal mucosa | Malignant ulcer | Moderately differentiated SCC | T3N0M0 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 13. | Jaythoon bee | 66 | F | - | - | -no habits | - | Right buccal mucosa | Malignant ulcer | Severe epithelial dysplasia-carcinoma in situ | T3N0M0 |
| 14. | Shakunthala | 65 | F | - | - | Tobacco powered with betel | 40yrs | Right lateral border tongue | Malignant ulcer | Well differentiated SCC | T3N2aM0 |
| 15. | Maheshwari | 55 | F | - | - | 10pkt/day | 10yrs | Right buccal mucosa | Malignant ulcer | Well differentiated SCC | T4N2bM0 |
| 16. | Malliga | 56 | F | - | - | 5pkt/day | 4 yrs | Right lateral border tongue | Malignant ulcer | Well differentiated SCC | T3N2aM0 |
| 17. | Banu | 48 | F | - - | - | 2pkt/day | 10yrs | Right buccal mucosa | Malignant ulcer | Well differentiated SCC | T4N0M0 |
| 18. | Maria john | 60 | M | 10/day | 15yrs | 6pkt/day | 15yrs | left lateral border tongue | Malignant ulcer | Well differentiated SCC | T3N0M0 |
| 19. | Raja | 48 | M | 5/day | 12yrs | 10pkt/day | 6yrs | left lateral border tongue | Malignant ulcer | Moderately differentiated SCC | T3N0M0 |
| 20. | Rani | 60 | f | - | - | - | - | Right buccal mucosa | Malignant ulcer | Well differentiated SCC | T2N0M0 |
| 21. | Murugesan | 65 | M | 20/day | 25yrs | - | - | palate | Malignant ulcer | Moderately differentiated | T3N2aM0 |
| 22. | Ganesan | 52 | M | - | - | 12pkt/day | 3yrs | Right buccal mucosa | Malignant growth | Well differentiated SCC | T3N2aM0 |
| 23. | Yasodha | 60 | F | - | - | Snuff dipping | 36yrs | Lower lip and left buccal mucosa | Malignant growth | Well differentiated SCC | T4N2aM0 |
| 24. | Parthasarathi | 58 | M | - | - | 3-4pkt/day | 23yrs | Right buccal mucosa and alveolar mucosa | Malignant growth | Well differentiated SCC | T4N2aM0 |
| 25. | veerappan | 60 | m | 25bidi | 35yrs | Snuff dipping | 40yrs | Right buccal mucosa | Malignant ulcer | Well differentiated SCC | T4N2aM0 |

## MASTER CHART GROUP III

| S.no | Name | AGE | SEX | Site | Clinical diagnosis | Diagnosis | TNM staging | Treatment | Duration Of Treatment |
|---|---|---|---|---|---|---|---|---|---|
| 1. | Munusamy | 62 | M | Right buccal mucosa | Malignant growth | Well differentiated SCC | $T_2N2aM0$ | Radiotherapy | 2nd week of Treatment |
| 2. | Umaya | 45 | F | Left buccal mucosa and mandible, skin of cheek | Malignant growth | Well differentiated SCC | T4N2aM0 | Radiotherapy And surgery | 2nd week of Treatment after surgery |
| 3. | Lakshmi | 48 | F | Gingivobuccal complex left side | Malignant ulcer | Well differentiated SCC | T3N0MO | Radiotherapy | 2nd week of treatment |
| 4. | Boopathi | 60 | m | Left buccal mucosa | Malignant growth | Well differentiated SCC | T2NoMo | Radiotherapy | 4th week of treatment |
| 5. | Gunaselan | 60 | M | Palate | Malignant ulcer | Moderately differentiated SCC | T2N0M0 | Radiotherapy | 2nd week |
| 6. | Mani | 65 | M | Right lateral border tongue | Malignant ulcer | Moderately differentiated SCC | T4N2bM0 | Surgery Radiotherapy | 3rd week |
| 7. | Ramachandran | 44 | M | Left buccal mucosa | Malignant ulcer | Well differentiated SCC | T4N2bM0 | Radiotherapy | 4th week |
| 8. | Srinivasan | 56 | M | Right lateral border tongue | Malignant ulcer | Well differentiated SCC | T3N0M0 | Radiotherapy And chemotherapy | 3rd week |
| 9. | Chellamal | 57 | F | Right buccal mucosa | Malignant ulcer | Well differentiated SCC | T3N0M0 | Radiotherapy and chemotherapy | 4th week |
| 10. | Gopal | 60 | M | lower lip and tongue | Malignant growth and leukoplakia tongue | Well differentiated SCC | T4N2bM0 | Palliative radiotherapy | 2nd week |
| 11. | Ramalingam | 41 | M | Right retromolar trigone and buccal mucosa | Malignant ulcer | Well differentiated SCC | T3N2aMO | Radiotherapy | 2nd week of treatment |

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|---|---|---|---|---|---|---|---|---|---|
| 12. | Mariammal | 56 | F | Right buccal mucosa | Malignant ulcer | Well differentiated SCC | T3N0MO | Radiotherapy | 2nd week of treatment |
| 13. | Saroja | 61 | F | Right lateral border tongue | Malignant ulcer | Well differentiated SCC | T3N0MO | Radiotherapy | 4th week |
| 14. | Vinayagi | 75 | F | Left buccal mucosa | Malignant ulcer | Well differentiated SCC | T4N2bM0 | Radiotherapy and chemotherapy | 2nd week of treatment |
| 15. | Manimegalai | 56 | F | Right lateral border tongue | Malignant ulcer | Well differentiated SCC | T3N2bMO | Radiotherapy | 3rd week |
| 16. | bhavani | 60 | F | Right lateral border tongue | Malignant ulcer | Well differentiated SCC | T4N2bM0 | Radiotherapy and chemotherapy | 4th week |
| 17. | Maria john | 60 | M | left lateral border tongue | Malignant ulcer | Well differentiated SCC | T3N0MO | Radiotherapy | 3rd week |
| 18. | suresh | 19 | M | left lateral border tongue | Malignant ulcer | Moderately differentiated SCC | T2N0M0 | Radiotherapy | 3rd week |
| 19. | Lakhsmi | 52 | f | Right buccal mucosa | Malignant ulcer | Well differentiated SCC | T3N2aMO | Radiotherapy | 2nd week |
| 20. | Manikandan | 29 | M | Right buccal mucosa | Malignant growth | Well differentiated SCC | T3N2bMO | Radiotherapy and chemotherapy | 4th week |
| 21. | Anil kumar | 26 | M | Right retromolar trigone and buccal mucosa | Malignant ulcer | Moderately differentiated SCC | T2N0M0 | Radiotherapy and chemotherapy | 4th week |
| 22. | Narayansamy | 73 | F | Right buccal mucosa | Malignant ulcer | Well differentiated SCC | T4N2bM0 | Palliative Radiotherapy | 2nd week |
| 23. | Pandidurai | 44 | m | Left buccal mucosa | Malignant growth | Well differentiated SCC | T3N0MO | Radiotherapy | 3rd week |
| 24. | Rajesekar | 51 | M | Floor of mouth And right buccal mucosa | Malignant ulcer | Well differentiated SCC | T4N2bM0 | Radiotherapy | 2nd week |
| 25. | Subramani | 80 | M | Left gingivo buccal complex | Malignant growth | Moderately differentiated SCC | T4N2aM0 | Radiotherapy | 4th week |

MASTER CHART –IV CONTROL GROUP

| S.NO | NAME | AGE | SEX |
|---|---|---|---|
| 1. | Prabakar | 24 | M |
| 2. | Manikandan | 23 | M |
| 3. | Suresh | 36 | M |
| 4. | Vanitha | 30 | F |
| 5. | Lokesh | 23 | M |
| 6. | Rajalaksmi | 32 | F |
| 7. | Kamal | 31 | M |
| 8. | Jagadeshwari | 42 | F |
| 9. | Archana | 22 | F |
| 10. | Usha | 30 | F |
| 11. | Arumugam | 51 | M |
| 12. | Prabu | 28 | M |
| 13. | Veeran | 26 | M |
| 14. | Metha | 40 | F |
| 15. | Farook bahsha | 45 | M |
| 16. | Arul | 52 | M |
| 17. | Velu | 56 | M |
| 18. | Savithri | 46 | F |
| 19. | Padma | 48 | F |
| 20. | Lakshmi | 34 | F |
| 21. | Raja | 46 | M |
| 22. | Jayanthi | 33 | F |
| 23. | Ambetkar | 28 | M |
| 24. | Devi | 28 | F |
| 25. | Vasantha sekar | 48 | M |